ALLEN'S
COMMERCIAL ORGANIC ANALYSIS

FIFTH EDITION, REWRITTEN, REVISED, RESET
COMPLETE IN TEN VOLUMES EACH VOLUME SOLD SEPARATELY

The organic chemicals and products employed in the arts, manufactures, commerce, medicine, science, etc. It treats upon the properties, modes of analysis, proximate analytical examination; methods for detection and estimation of impurities, adulterations, products of decomposition, etc.

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Introduction. By W. A. DAVIS, B. Sc., A. C. G. I., Rock Ferry, Cheshire, Eng. Preliminary Examination; Specific Gravity; Changes in Physical State; Optical Properties; Spectrometers and Spectrographs; Polarimeters; Arrangements for Maintaining a Known Constant Temperature; Ultimate Analysis; Moisture, Crude Fibre and Ash; Action of Solvents.

Alcohols. By L. M. BURGHART, M. A., Baltimore, Md. Methyl Alcohol; Wood Naptha; Crude Wood Spirit; Acetone; Ethyl Alcohol; Higher Aliphatic Alcohols.

Malt and Malt Liquors. By JULIAN L. BAKER, F. I. C., Staines, Eng. Malt; Malt Worts; Roasted Barley and Malt; Brown and Crystal Malts; Malt Substitutes; Grits and Raw Grain; Malt Extract; Caramel; Invert Sugar; Starch Sugars; Preparation of Materials; Beer and Ale.

Wines and Potable Spirits. By LEWIS EYNON, B. Sc., F. I. C., London. Wines; Significance of Results of Wine Analysis; Cider; Potable Spirits.

Yeast. By EMIL SCHLICTING, PH. D., New York. Yeast; Culture Yeast; Pure Culture of Yeast and Its Application in Practice; Physical Examination of Yeast.

Neutral Alcohol Derivatives. By HENRY LEFFMANN, M. D., PH. D., Philadelphia. Ether; Aldehydes; Method of Determining Chloroform in Medicinal Preparations.

Sugars. By LEWIS EYNON, B. Sc., F. I. C., London. Classification; Methods of Analysis Depending on Specific Gravity or Solution Density; Methods of Analysis Depending on Optical Activity; Method of Analysis Depending on Refractive Index; Methods of Analysis Depending on Reducing Power; Method of Analysis Depending on Oxidation with Iodine; Methods of Analysis Depending on Fermentation; Cane Sugar, Analysis and Valuation of Cane and Beet Sugar Products; Sucrose in Beetroot; Maltose; Lactose; Monosaccharides; Honey; Maple Products; Urine Analysis; Pentoses.

Starch and Its Isomerides. By T. H. POPE, B. Sc., F. I. C., Wallasey, Cheshire, Eng. Starch; Estimation of Starch; Dextrine, Amylin; Cellulose; Gums;

Proximate Analysis of Plants; Cereals; Wheat, Flour; Bread; Macaroni; Vermicelli, Spaghetti, Noodles, etc., Biscuits and Milk Flour; Other Cereals.

Paper and Pulp Testing. By E. SUTERMEISTER, S. B., Westbrook, Me. Paper; Physical Tests; Chemical Tests; Wood Pulp.

Aliphatic Acids. By HUGO SCHLATTER, M. S., Wilmington, Del. General Reactions; Acetic Acid; Vinegar; Homologues of Acetic Acid; Malic Acid; Tartaric Acid; Tartrates; Citric Acid; Citrates; Lactic Acids.

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Special Characters and Modes of Examining Fats, Oils and Waxes. By C. AINSWORTH MITCHELL, M. A., F. I. C., London. Olive Oil Group; Rape Oil Group; Cottonseed Oil Group; Linseed Oil Group; Castor Oil Group; Cacao Butter Group; Lard Oil Group; Tallow and Butter Group; Whale Oil Group; Sperm Oil Group; Beeswax Group.

Butter Fat. By CECIL REVIS and E. R. BOLTON, London. General; Qualitative Tests; Butter; Estimations; Preservatives; Margarine; Hardened Fats; Vitamines; Ghee.

Lard. By GEORGE A. REITZ, B Sc., PH. C., Philadelphia.

Linseed Oil. By GLENN H. PICKARD, Minneapolis. General; Iodine; Hexabromide Text; Other Tests and Methods, Oxygen Absorption; Polymerised Oil; Refining; Air Treated Oils; Boiled Oil; Effect of Storage; Detection of Adulterants.

Higher Fatty Acids. Revised by H. E. COX, M. Sc., PH. D., F. I. C., Newport. Characteristics; General Properties; Separation of Mixed Fatty Acids; Palmitic Acid; Stearic Acid; Oleic Acid; Sebacic Acid; Elaidic Acid; Sulpholeic Acid.

Soaps. By ELBERT C. LATHROP, A. B., PH. D., Philadelphia. General; Detergent Action; Raw Materials; Alkalies and Fillers Varieties; A. C. S. Methods; Separation of Unsaponified Matters; Phenols; Examination for Special Constituents; Soap Powders, etc.; Interpretation of Analyses; Specifications.

Glycerin. By J. W. LAWRIE, PH. D., Wilmington. Uses for Glycerin; Pharmacopoeia Test; Qualitative Test; Estimation; Specific Gravity; Refractive Index; Boiling Points; Analysis of Crude Glycerin; Analysis of Refined Glycerin; Pure Glycerin; Moisture in Glycerin; Foots; Polyglycerins; Fermentation Glycerin; Glycerin in Wines; Physical Constants.

Wool-fat, Wool-grease, Suint, Degras. By AUGUSTUS H. GILL, PH. D., Sc. D., Boston. Wool-fat; Quantitative Methods; Lanolin; Distilled Wool-grease; Degras; Cloth Oils.

Sterol Alcohols. By JOHN ADDYMAN GARDNER, M. A., F. I. C., London. Cholesterol; Vegetable Sterols; Sources of Sterols; Phytosterol; Amorphous Sterols; Extraction of Sterols; Colorimetric Methods.

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Bitumens. By SAMUEL P. SADTLER, PH. D., L. L. D., Philadelphia. Natural Gas; Petroleum; Distillation of Petroleum; Naphtha; Kerosene; Gas Oil; Lubricating Oils; Lubricating Greases; Petrolatum; Paraffin; Asphalt; Asphalt Fluxes; Asphalt Pavings; Roofing Papers; Bibliography.

Napthalene and Its Derivatives. By W. A. DAVIS, B. Sc., A. C. G. I., Rock Ferry. Napthalene; Napthalene Oils; Napthols; Napthol Ethers; Napthol Sulphonic Acids; Bibliography.

Anthracene and Its Associates. By JOHN H. SACHS, PH. D., Wilmington. Anthracene; Anthraquinone; Phenanthrene; Carbazol; Compounds with Picric Acid; Valuation of Anthracene; Bibliography.

Phenols. By J. BENNETT HILL, PH. D., Philadelphia. Monohydric Phenols; Phenol; Cresols; Xylenols; Commercial Carbolic Acids; Dip and Flotation Oils; Creosote; Cresylic Acid Disinfectants; Dihydric Phenols; Guaiacol; Wood Creosote; Trihydric Phenols; Bibliography.

Aromatic Acids. By EDWARD HORTON, B. Sc., London. Sulphonated Phenols; Napthol Sulphonic Acids; Benzoic Acid; Metallic Benzoates; Benzoic Esters; Benzoic Aldehyde; Oil of Bitter Almonds; Saccharin; Cinnamic Acids; Cinnamic Esters; Cinnamic Aldehydes; Oil of Cinnamon; Coumarin; Gum Benzoin; Peruvian Balsam; Tolu Balsam; Liquid Storax; Salicylic Acid; Metallic and Alkaloidal Salicylates; Salicylic Esters; Derivatives of Salicylic Acid; Homologues of Salicylic Acid; Hydroxy-toluic Acids; Dihydroxy-benzoic Acids; Vanillin; Bibliography.

Gallic Acid and Its Allies. By W. P. DREAPER, O. B. E., F. I. C., London. Gallic Acid; Esters and Derivatives of Gallic Acid; Pyrogallol; Bibliography.

Phthalic Acid and the Phthaleins. By W. A. DAVIS, B. Sc., A. C. G. I., Rock Ferry. Phthalic Acids; Phthalic Anhydrides; Phthaleins; Phenolphthalein; Indicators; Bibliography.

Modern Explosives. By A. MARSHALL, F. I. C., Kirkee, India. Introductory; Cellulose Nitrates; Examination of Nitrocellulose; Nitrostarch; Nitroglycerin; Separation of Nitro Aromatic Compounds; Picric Acid;

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Resins. By ERNEST J. PARRY, B. Sc., F. I. C. Chemical Composition; Resin Esters; Resin Alcohols; Resinolic Acids; Resenes; Characters of Resins; Commercial Resins; Acaroid; Amber; Colophony; Common Resin; Colophonates; Dry Distillation; Rosin Spirit; Rosin Oil; Detection of Adulterants; Copals; Sandarac; Mastic; Dammar Resin; Dragon's Blood; Guaiacum; Shellac; Oleoresins; Balsams; Common Turpentine; French Turpentine; Venice Turpentine; Larch Turpentine; Canada Balsam, Balsam of Copaiba; Copaiba; Capivi; Oleoresin of Cubebs; Capsicin; Gum Resins; Ammoniacum; Asafoetida; Elemi; Myrrh; Bdellium; Gamboge; Frankincense; Sagapenum; Ambergris; Civet; Castor; Scammony Resin; Podophyllum Resin; Jalap Resin.

India Rubber, Gutta-Percha, Balata and Allied Substances. By JOHN B. TUTTLE, B. Sc. Polyterpenes; India-Rubber; Rubber Substitutes; Reclaimed Rubber; Gutta-Percha.

Constituents of Essential Oils and Allied Substances. By ERNEST J. PARRY. B. Sc., F. I. C. Hydrocarbons; Pentines; Hemiterpenes; Cymene; Terpenes; Monocylic Terpenes; Limolene; Terpinolene; Terpinene; Phellandrene; Sylvestrene; Dicyclic Terpenese; Pinene; Camphene; Borynlene; Fenchene; Thujene; Carene; Olefinic Terpenes; Constitution of the Dicyclic Terpenesene; Sesquipenes; Cadinene; Caryophyllene; Santalene; Gurjunene; Zingiberene; Diterpenes and Polyterpenes; Estimation of Hydrocarbons; Alcohols; Alcohols of the Methane and Allied Series: Geraniol

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General Character and Analysis of Essential Oils. By Ernest J. Parry, B. Sc., F. I. C. Extraction of Essential Oils, Composition; General Characters; Analysis of Essential Oils; Detection of Sulphur Compounds in E. O.; Estimation of Free Acids in E. O., Estimation of Alcohols in E. O.; Estimation of Esters; Aldehydes· Ketones; Carbonyl Number; Iodine Value of Essential Oils; Methoxyl Values; Optical Activity; Refractive Indices; Adulteration of Essential Oils.

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Writing, Stamping, Typing and Marking Inks. By C. AINSWORTH MITCHELL, M. A., F. I. C., England. Inks; Examination of Writing Inks; Valuation of Writing Inks; United States Government Master Specification for Writing Ink; for Record and Copying Ink; for Red Ink; for Hectograph Ribbons; Modern Carbon Writing Inks; Chemical Examination of Ink in Writing; Type Writing Inks; Typewriter Ribbons; United States Government Master Specification for Typewriter Ribbons, for Ribbons for Computing and Recording Machines; Carbon Papers; Rubber-Stamp Inks; Marking Inks.

Printing Inks. By JOHN B. TUTTLE, B. Sc., New York. Types of Inks; Composition; Manufacture of Ink; Analysis; Separation of the Oil; Oil Constants; Hard Gums; Unsaponifiable Matter; Analysis of the Pigment; Blue Inks; Red Inks; Green Inks; Inks of Other Colours; Permanence to Light; Dyes and Lakes; Special Tests.

Amines and Ammonium Bases. By H E. COX, M. Sc., PH. D., F. C. I., England. Monamines; Hydrazines; Substituted Hydrazines.

Analysis of Leather. By A. E. CAUNCE, M. Sc., F. I. C., England. Physical Tests; Analysis of Vegetable-Tanned Leather; Analysis of Chrome Leather.

Colouring Matters of Natural Origin. By PROF. W. M. GARDNER, M. Sc., F. I. C., England. Indigo; Logwood; Natural Yellow Colouring Matters; Fustic; Weld; Turmeric; Gamboge; Saffron; Annatto; Red Dyestuffs; Orchil and Cudbear; Litmus; Madder; Redwoods; Alkanet; Safflower; The Colouring Matters in Flowers.

Colouring Substances in Foods. By WALTER E. MATHEWSON, Topeka, Kansas. Qualitative Examination of Food Products for Pigments; General Methods for the Qualitative Separation or Isolation of Soluble Colouring Matters; Identification of Colouring Substances; Quantitative Spectro-Colorimetry; Analysis of Commercial Food Colouring Materials.

Benzene and Its Homologues. By J. BENNETT HILL, PH. D., Philadelphia. Properties of Benzene Hydrocarbons; Commercial Benzene; Thiophene; Toluene; Xylenes; Trimethylbenzenes. Para-Methyl-isopropyl Benzene: Commercial Benzols; Coal Tar Light Oils.

Aniline and Its Allies. By A. B. DAVIS, Cincinnati, Ohio. Aniline; Salts of Aniline; Aniline-Sulphonic Acids; Homologues of Aniline; Sulphonic Acids; Homologues of Aniline; Analysis of Commercial Xylidine (Flotation Grade); Aniline Oils; Estimation of Acetanilide and Phenacetin in Admixture; Commercial Method for the Analysis of Meta-Toluylene-Diamine; Method for the Analysis of Benzidine; for the Analysis of Tolidine; for the Analysis of Dianisidine.

Naphthylamines, Pyridine, Quinoline and Acridine Bases. By A. B. DAVIS, Cincinnati; Ohio. Naphthylamines and Their Allies; Alkyl- and Acyl-Naphthylamines; Pyridine Bases; Acridine and Its Allies.

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Dyes and Colouring Matters. By HANS EDWARD FIERZ-DAVID, D. Sc., Zurich, Switzerland. Chemical Identification of the Different Groups; Historical Outline; Relations of Colouring Matters to Fibres; Classification of Dyes and Colouring Matters; Index of Names of Firms; Nitroso Colouring Matters and Nitro-colouring Matters; Azo Coloring Matters; Groups of Azo Dyes; Azo-colouring Matters. Mono-azo-dyes; Disazo Dyes; Primary Disazo Dyes; Secondary Disazo Dyes; Diamine Colours; Trisazo Dyes of Different Constitution; Tetrazo Dyes of Different Constitution; Stilbene Colouring Matters; Pyrazolone-dyes; Carbonium-dyes; Diphenylmethane Dyes; Triphenylmethane-dyes; Xanthenes; Acridine Dyes; Quinoline Dyes; Thiazoles (Primulines); Indamines, Indoanilines and Indophenols; Azines; Oxazine Colouring Matters; Thiazines; Sulphur Colouring Matters; Hydroxy-ketone Dyestuffs; Hydroxy-anthraquinones; Acid Anthraquinone Dyes; Anthraquinone Vat Colours; Indigoid Colouring Matters.

The Synthetic Dyestuffs. By A. W. JOYCE, PH. D., New York. Classification of the Synthetic Dyestuffs; The Nitroso Dyestuffs; The Nitro Dyestuffs; The Azo Dyestuffs; The Basic Azo Dyes; Acid Monazo Dyes; Monazo Dyes from Ortho-aminophenols; The Ice Colours; Pyrazoline Dyes; Acid Disazo Dyes; Substantive or Direct Cotton Dyes; Direct Cotton Dyes Derived from Diamines of the Benzene and Naphthalene Series; Direct Cotton Dyes Derived from J-acid and Its Derivatives; Dyes from Thiazole Bases; The Stilbene Dyes; Ketonimines; The Carbonium Colouring Matters; The Arylmethane Dyes; Diamino Derivatives; Triamino Derivatives; Amino-oxy-derivatives; Oxy-derivatives; Diphenyl-naphthyl Methane Dyes; Xanthene Dyes; The Amino Derivatives; The Hydroxy Derivatives; Amino-hydroxy Derivatives; Acridine Dyes; Quinoline Dyes; Thiazole Dyes; Indophenols and Indamines; Oxazine Dyes; Thiazine Dyes; Azine Dyes; Sulphur Dyes; Anthraquinone Dyes; Mordant-dyeing Anthraquinone Dyes; Anthraquinone Acid Dyes; Vat Dyes; Indigo and Indigoid Vat Dyes; Benzoquinone Vat Dyes; Identification of Azo Dyes; Reduction Products of Azo Dyes and the Dyes from Which They are Obtained; Physical and Chemical Properties of the Reduction Products from Azo Dyes.

Analysis of Colouring Matters. By HANS EDWARD FIERZ-DAVID, D. Sc., Zurich, Switzerland and V. E. YARSLEY, D. Sc., M. Sc., A. I. C., General; Colour Standards and Colour Comparison; Aniline Lakes; Spectroscopic Investigation; Qualitative Investigation of Dyestuffs in Substance; Qualitative Investigation of Dyestuffs on Animal Fibre; Triphenylmethane Dyestuffs; General Procedure; Treatment of Mixtures; Qualitative Investigation of Dyestuffs on Vegetable Fibres; Preliminary Investigations; Chemical Reactions of the More Important Classes of Dyestuffs; Quantitative Analysis of Dyestuffs; Relative Methods; Absolute Methods; Titration with Hydrosulphite; Bibliography.

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General Section on Alkaloids. By T. M. SHARPE, M. Sc. TECHN. A. I. C. London. Alkaloids of Alstonia Bark; Alkaloids of Areca or Betel Nut; Alkaloid of Barley Germs; Alkaloids of the Common Broom; Alkaloids of the Calabar Bean; Alkaloids of Delphinium; Alkaloids of Ephedra Spp.; Alkaloids of Ergot; Toxicological Investigation of Ergot; Alkaloids of Hemlock; Poisoning by Coniine and Hemlock; Assay of Hemlock and its Preparations; Alkaloids of Holarrhena Spp.; Alkaloids of Ipecacaunha; Alkaloids of Jaborandi; Alkaloids of Yellow Jasmine; Alkaloid of Laburnum and Furze; Alkaloids of Lobelia Inflata; Alkaloids of Lupuis; Alkaloids of Meadow Saffron (Colchicine); Assay of Colchicum; Toxicology of Colchicum; Alkaloids of Mescal Buttons; Alkaloid of Papaya; Alkaloids of Peganum Harmala; Alkaloids of Yagé and Caapi; Alkaloids of Pepper; Analysis of Pepper; Alkaloids of Pomegranate; Alkaloids of the Potato, etc.; Alkaloids of Labadilla; Alkaloids of the Hellebores (Veratrum); Alkaloid of Yew; Alkaloids of Yolumba Bark; Alkaloids of Aspidosperma Quebracho Blanco; Bibliography.

Aconite Alkaloids. By FRANCIS H. CARR, C. B. E., F. I. C., London. Species of Aconite Plants; Constitution and Characters of the Aconite Bases; Aconitine; Salts of Aconitine; Chemical Reactions of Aconitine; Derivatives of Aconitine; Benzaconine; Aconine; Pyraconitine; Pyraconine; Amorphous Alkaloids of A. Napellus; Japaconitine; Indaconitine; Pseudo-aconitine; Bikhaconitine; Jesaconitine; Lycaconitine and Myoctonine; Lycoctonine; Myoctonine; Lapaconitine, Leptentrionaline and Cynoctine; Atisine; Assay of Aconite Root and its Preparations; Toxicology of Aconite; Toxicological Detection of Aconite; Pharmacology of Aconite.

Berberine and Its Associates. By E. HORTON, B. Sc. Berberine; Constitution of Berberine; Reactions and Detection of Berberine; Estimation of Berberine; Volumetric Methods; Gravimetric Method; Salts of Berberine; Oxyacanthine; Berbamine; Hydrastine; Estimation of Hydrastine; Hydrastis Rhizome; Hydrastinine; Salts of Hydrastinine; Canadine; Calumbin.

Caffeine, Tea and Coffee. By J. J. FOX, D. Sc., F. I. C. and P. J. SAGEMAN, F. I. C., London. Caffeine and its Allies; Caffeine, Theine or Trimethylxanthine; Salts of Caffeine; Assay of Caffeine Sodium Salicylate; Theobromine; Diuretin; Derivatives of Caffeine; Theophylline; Tea; Constituents of Tea; Analysis of Tea; Moisture in Tea; Ash; Isolation and Estimation of Caffeine; Tannin; Extract; Stalks; Essential Oil; Adulterations of Tea; Caper Tea; Maté Paraguay Tea; Coffee; Composition of Coffee; Caffetannic Acid; Caffeol; Coffee Berries; Analysis of Coffee and Coffee Mixtures; Coating and Glazing Substances; Ground Coffee; Commercial Chicory; Coffee Extracts; Kola; False Kola or Kola Bitter; Guarana; Bibliography.

Cinchona Alkaloids. By OLIVER CHICK, F. I. C., London. Cinchona Barks; Composition of Cinchona Barks; Assay of Cinchona Barks; Separation of Cinchona Bases; Titration of Cinchona Alkaloids; Cinchona Alkaloids; Constitution; General Properties of Cinchona Bases; Quinine; Detection and Estimation of Quinine; Salts of Quinine; Examination of Commercial Quinine Sulphate; Iron and Quinine Citrate; Tincture of Quinine; Quinine Tablets; Hydroquinine; Quinidine; Quinamine; Cinchonidine; Cinchonine; Amorphous Cinchona Bases; Alkaloids of Remijia Bark; Homoquinine; Bibliography.

Cocaine. By SAMUEL P. SADTLER, Philadelphia. Revised by NORMAN EVERS, B. Sc., F. I. C., London. Cocaine; Qualitative Tests; Toxiocological Identification of Cocaine; Separation and Determination of Cocaine; Salts of Cocaine; Examination of Commercial Cocaine and its Salts; Decomposition Products of Cocaine; Bases allied to Cocaine; Cocaine Substitutes; Coca Leaves; Liquid Extract of Coca.

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WITH CONCISE METHODS FOR

THE DETECTION AND ESTIMATION OF THEIR IMPURITIES, ADULTERATIONS, AND PRODUCTS OF DECOMPOSITION

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PREFACE

The arrangement in this volume is somewhat different from what it was in the Fourth Edition. It is, in effect, a preparation for Volume VI, on Coal Tar Colours. A section on Benzene and Its Homologues has been introduced here rather than in Volume III, as it may be looked upon as a first step on the road to coal tar dyes, and should be considered before dealing with the intermediates. Natural dye-colours are used considerably as foundation materials for the after-treatment with coal tar colours. Naturally, tanning materials and inks should be considered in conjunction with natural colours which are rich in tannin content. The synthetic dyes will be fully treated in Volume VI.

Many of the sections have been entirely rewritten, and the remain der fully revised.

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TANNINS

BY M. NIERENSTEIN, D. Sc., Ph. D.

DESCRIPTION AND GENERAL PROPERTIES OF TANNINS

The Tannins are amorphous or crystalline solids of astringent taste, more or less soluble in water, freely so in alcohol, or a mixture of alcohol and ether, and notably in ethyl acetate. They are almost insoluble in dry ether, and quite so in chloroform, benzene, petroleum spirit, and carbon disulphide. Their aqueous solutions give blue-black or green colorations or precipitates with ferric salts, and are precipitated by the acetates of lead and copper and by stannous chloride. Most tannins are precipitated by tartar emetic and by mineral acids. In some cases tannin combines with the base only, but in others, as when cupric acetate is employed, the salt is said to enter into combination as a whole. With a solution of gelatin, the tannins give precipitates, similar to those obtained with leather, which are insoluble in the presence of excess of tannin or salts but not wholly insoluble in pure water.[1] The majority of tannins can be completely removed from their aqueous solutions by the introduction of rasped skin or hide. The tannins are also removed from solution by digestion with cupric or zinc oxide, and they reduce Fehling's solution on heating. The tannins give insoluble precipitates with many organic bases, the cinchonine compounds being among the least soluble.

The most characteristic and commercially useful properties of the tannins are the formation, by combination with gelatin and gelatin-forming tissues, of the insoluble compounds which constitute leather; and the formation of lakes.

The natural tannins are powerful reducing agents, and exhibit a marked tendency to absorb oxygen, especially in alkaline solution. The oxidation-products are strongly coloured.

[1] For a very interesting summary of this question see: A. W. Thomas and M. W. Kelly. *J. Ind. Eng. Chem.* 1924, **16**, 31.

Extraction of Tannins.—The different natural tannins exhibit such differences in their chemical reactions and behaviour with solvents that it is not possible to give a general rule for their preparation in a state of purity.

The method of Pelouze (*Ann. Chim. Phys.* 1834, **54**, 337) for the preparation of gallotannin from gall-nuts is as follows: The powdered substance is exhausted with ordinary ether containing alcohol and water. On standing, the liquid separates into two layers, the lower of which contains the gallotannic acid whilst the upper ethereal layer retains the gallic acid. The tannin obtained by separating and evaporating the lower layer may be further purified by dissolving it in water and adding to the solution twice its volume of ether, when three layers are formed, the lowest of which contains nearly pure gallotannin. This solution is drawn off, and magnesium sulphate is added to remove the excess of water. The ethereal solution is then evaporated to dryness.

A useful process of extracting tannins is the following: The finely divided substance is exhausted by treatment with rectified spirit, the solution filtered and evaporated to a small bulk at as low a temperature as possible (preferably under reduced pressure). The extract is treated at once with a considerable proportion of cold water, the liquid filtered and fractionally precipitated with lead acetate. The first and last fractions should be rejected, as they usually contain colouring matters and other foreign substances. The tannate of lead is washed as rapidly as possible, suspended in water and decomposed by hydrogen sulphide. Although generally recommended, it must be noted that hydrogen sulphide decomposes gallotannin (Nierenstein, *Ber.*, 1909, **42**, 3552). The filtrate is shaken with ether to remove gallic acid, separated from the ethereal layer, and evaporated in a partial vacuum to the consistence of a thin syrup. The remaining water should be removed by exposure over sulphuric acid at the ordinary temperature.

Von Schroeder and Bartel (*Dingler's J.*, 1896, **289**, 113) investigated the effect of prolonged boiling in the extraction of tannins. They considered that if a sufficient volume of water is used, only a few hours of boiling are necessary to extract the largest part of the tannin, succeeding extractions only removing traces of tannin from the residue. Prolonged boiling extracts an excess of non-tannins; therefore it is not advisable to push the extraction too far, as the

quality of the whole product will be deteriorated by the excess of non-tannin constituents.

Parker (*J. Soc. Chem. Ind.*, 1899, **28**, 106) showed that by extracting for a prolonged time at a low temperature, and afterwards at a higher temperature, it is possible to fractionate the different tannins. Between 60° and 90° seems to be the optimum temperature for the extraction of tannins under ordinary conditions.

Many tannins (*e. g.*, sumach tannin and ratanhia tannin) may be purified by shaking with ether to remove gallic acid, saturating the concentrated aqueous solution with common salt, and shaking it with ethyl acetate which then removes the tannin.

Some tannins (*e. g.*, hop tannin and alder tannin) are stated to be insoluble in water after isolation, the change being probably due to change in constitution. When the presence of such tannins is suspected, the lead precipitate should be suspended in alcohol instead of water, before decomposing it with hydrogen sulphide.

Many other vegetable substances besides tannins are precipitable by lead acetate, but they are generally insoluble in cold water.

It sometimes happens that a single plant contains two or more tannins. Thus both oak-bark and willow-bark contain a little gallotannin in addition to their own peculiar tannins; myrobalans and divi-divi contain both gallotannin and ellagitannin. The existence of several tannins may be detected in some cases by fractional precipitation with lead acetate, and in others by examining the products of the action of dilute acid. Thus, it is stated that oak-red (phlobaphene) produced from quercitannin is not removed by agitating the liquid with ether, whilst the gallic acid produced from the gallotannin is dissolved in this solvent.

Trimble (*The Tannins*, Vol. I, p. 87) employed acetone as a solvent, the process being a simple and satisfactory one. The powdered material was generally macerated for 48 hours in a percolator. The solvent was then run off, 500 c.c. being used for each 1000 grm. of tannin material in a fine state of division. The acetone was removed by evaporation, the residue dissolved in water or alcohol, and the solution filtered and diluted with water until the anhydrides and colouring matters were thrown out of solution.

In some cases crystalline tannins have been obtained by more or less the same methods. These crystalline tannins are: (1) Chebulic acid (A. Fridolin, *Diss.* Dorpt 1884, W. Adolphi, *Diss.* Dorpt,

1893; Freudenberg, *Ber.*, 1919, **52**, 1238, (2) Hamameli-tannin (Grüttner, *Diss.* Berlin, 1898, Freudenberg, *Ber.*, (1919, **52**, 177; Nierenstein, unpublished work), (3) Gluco-gallin (Gilson, *Bull. Acad. Roy. méd. de Belgique*, 1902, (iv) **16**, 842; Feist, *Chem. Zeitg.* 1908, **32**, 918; *Ber.*, 1912, **45**, 1493; *Arch. d. Pharm.* 1913, **250**, 668; **251**, 468; Fischer and Bergmann, *Ber.*, 1918, **51**, 1760, (4) Paullinia-tannin (Nierenstein, *J. Chem. Soc.*, 1912, **121**, 23); and (5) Acertannin (Perkin and Uyeda, *J. Chem. Soc.* 1922, **121**, 66).

Many methods for the purification of the tannins have been described, but it is very doubtful if any of these methods really yields a uniform product. This is particularly evident from the recent investigations which have been published by Karrer. (*Acta Chim. Helv.*, 1923, **6**, 1; *Annalen*, 1923, **433**, 288.) It is, therefore, not proposed to give these methods in detail, but reference should be made to: Nierenstein, "*Chemie der Gerbstoffe*" (1910) and Freudenberg, "*Die Chemie der Natürlichen Gerbstoffe*" (1920).

CLASSIFICATION AND CONSTITUTION OF NATURAL TANNINS

From the tanner's point of view, the natural tannins are arranged in two general classes, namely, those which produce a "bloom," or fawn-coloured deposit, on leather, and those which do not. The tannins giving a "bloom" to leather give a blue-black coloration with ferric acetate, whilst the others afford a green colour with the same reagent. To the first of these classes belong the tannins of gall-nuts, myrobalans, divi-divi, sumach, valonia and oak-bark, whilst the second includes the tannins of catechu, hemlock, larch, ratanhia, mangrove, and all the varieties of mimosa. The production of the "bloom" is due to the formation of ellagic acid, $C_{14}H_6O_8$, a substance allied to gallic acid, $C_7H_6O_5$. The tannins of oak-bark and valonia are either mixtures of two distinct tannins or are of unknown constitution, for they yield both gallic acid and protocatechuic acid.

The arrangement of natural tannins into classes is therefore based on the products they yield: (1) when heated alone, (2) when heated with dilute acid, and (3) when fused with alkali hydroxide. The characteristic products obtained by heating tannins alone are pyrogallol and catechol; by heating with dilute acids, dextrose, gallic acid, ellagic acid, and insoluble amorphous anhydrides called phloba-

phenes; and by fusion with alkali hydroxide, pyrogallol, protocatechuic acid, acetic acid, and phloroglucinol.

Certain of the tannins give a blue or black coloration when mixed in solution with ferric salts, whilst others yield a green or greenish colour when similarly treated. Speaking generally, the tannins which are derived from gallic acid give a blue indication, whilst those derived from protocatechuic acid afford a green colour.[1]

The behaviour with iron salts is best observed by adding to an aqueous solution of the tannin contained in a test-tube one or two drops of dilute solution of ferric acetate. This may be prepared by adding sodium acetate to a solution of ferric chloride. Excess of the reagent must be carefully avoided, or its colour and oxidising action may lead to error. The coloration produced by ferric acetate having been observed, it is advisable to add an excess of ammonia, and note any change which may occur.

If ferric chloride be substituted for the acetate, the general results are the same, but in some instances a greenish coloration is produced by tannins which give a distinct blue colour with ferric acetate. This is especially the case if the ferric chloride solution contains free acid. Hence the acetate is to be preferred as a reagent for tannins.

There have been numerous classifications suggested for the tannins (Compare, J. Dekker "*Die Gerbstoffe*" pp. 386–408) none of which, however, are of any actual validity. Reference must, however be made to the Stenhouse-Procter, the Perkin-Everest and the Freudenberg classifications.

The Stenhouse-Procter Classification: Group I.—Tannins which give a bluish colour with ferric chloride yield, when treated with alkali hydroxides, pyrogallol, and form on the surface of the tanned skin the so-called "bloom" (ellagic acid).

Group II.—Tannins which give a green colour with ferric chloride, with bromine water marked precipitates, yield with alkali hydroxides pyrocatechol derivatives and form the so-called reds (phlobaphenes).

Group III.—A small group which gives with ferric chloride a bluish-green colour, and with bromide water an ill-defined precipitate, yielding little "phlobaphenes," but a marked "bloom."

[1] Compare, however, Nierenstein, *J. Chem. Soc.* 1919, **115**, 1174, where it is shown that Knopper tannin gives a green coloration with ferric chloride, although it contains a pyrogallol nucleus. Similar observations have been made by: Stenhouse, *Phil. Mag.* (III) 1843, **23**, 335; Eisfeld, *Annalen* 1854, **92**, 109; 1859, **111**, 217; Bjalobreshesky, *Russ. Pharm. J.* 1900, **22**, 3; Iljin, *Diss.* Petersburg (1905).

The Perkin-Everest Classification: (I) *Depside*-tannins containing the -CO.O-group. To this group belong the gallotannins.

(II) Diphenylmethylolid-tannins, containing the $\begin{array}{ccc} & CO\text{-}O & \\ \vert\diagup & & \diagdown\vert \\ C_6H_4 & \text{---}\cdots & C_6H_4 \end{array}$ group. To this group belong the ellagitannins.

(III) Phlobatannins; these tannins yield phlobaphenes. This group represents the old catechol tannins.

The Freudenberg Classification: (I) Hydrolysable tannins in which the benzene nuclei are united to larger complexes through oxygen atoms.

(II) Condensed tannins, in which the nuclei are held together through carbon linkages.

Where both kinds of compounds are present in the molecule, *e. g.*, in ellagic acid, the classification is decided according to the genetic connection with other tannins.

Group I embraces (*a*) esters of phenolcarboxylic acids with each other or with other oxyacids (depsides), (*b*) esters of phenolcarboxylic acids with polyatomic alcohols and sugars (tannin class), and (*c*) glucosides. The most important criterion for Group I is hydrolysis to simple components by enzymes, particularly tannase or emulsin.

Tannins of Group II are not decomposed to simple components by enzymes.[1] They are generally, but not always, precipitable by bromine, and when treated with oxidising agents or strong acids condense to high molecular amorphous tannins or "reds." They are divided into two classes according to whether or not phloroglucinol is present. With the exception of some simpler ketones, oxybenzophenones and oxyphenylstyrylketones, the catechins belong to the phloroglucinol class, *e. g.*, the very important quebracho and, probably, oak tannin are in this class.

DISTINCTION BETWEEN ALCOHOLIC AND AQUEOUS TANNINS

This distinction is of importance, as in some countries a duty is only collected on alcoholic tannin. A tannin which has been pre-

[1] Freudenberg's assumption that enzymes do not hydrolyse the tannins of Group II is based on the observation that tannase, prepared by growing *Aspergillus niger* in gallotannin does not decompose the catechol tannins (Freudenberg's tannins of Group II). This has, been confirmed by Nierenstein (*Biochem. J.*, 1922, 16, 514), who has, however shown that a tannase may be obtained by growing the fungus in a solution in which gallotannin is replaced by the catechutannin from cube-gambier. This tannin hydrolyzes catechutannins but has no effect on gallotannin. We have, therefore, to distinguish between two kinds of tannase (gallotannase and catechutannase) which hydrolyse Freudenberg's tannins of Groups I and II, respectively.

pared by extraction with water, on treatment with ether and a subsequent evaporation of the ethereal extract, leaves a residue which, redissolved in alcohol, does not precipitate on the addition of water. On the contrary, a tannin which has been prepared by extraction with alcohol, gives under the same conditions a distinct precipitate, due to the fats and resins which accompany it.

The following is a description of the best methods of formation and recognition of the decomposition products obtained by the action of heat, dilute acids, and fused potassium hydroxide on different kinds of tannin.

GENERAL BEHAVIOUR OF TANNINS: ACTION OF HEAT

When a tannin which produces a "bloom" on leather is cautiously heated to about 200°, it is decomposed, with volatilisation of pyrogallol in feathery crystals. On the other hand, the tannins which produce no bloom, but red deposits, show a somewhat similar behaviour, but the sublimate consists of catechol. From oak-bark and valonia, which apparently contain a mixture of both kinds of tannin, and hence yield both "bloom" and red colouring matters, both catechol and pyrogallol are produced on heating.

In using the heating test for distinguishing the two classes of tannins, the temperature must be carefully regulated, or further changes take place, and the recognition of the pyrogallol or catechol will be complicated by the formation of metagallic acid and other secondary products. A better result is obtained by mixing the substance with several times its weight of sand or powdered pumice, and passing a stream of coal gas or carbon dioxide through the retort, so as to carry the products quickly out of the sphere of action. A still better and more convenient plan is the following, based on an observation of T. E. Thorpe (*Chem. News*, 1881, **43**, 109). About 1 grm. of the sample should be heated with 3 c.c. of pure glycerin to a temperature of 190° to 200° for 20 minutes. After cooling, the product is treated with about 20 c.c. of water, and the liquid shaken with an equal volume of ether without previous filtration. The ethereal layer, which contains the pyrogallol and catechol, is separated from the aqueous liquid, evaporated to dryness and the residue dissolved in 50 c.c. of warm water. The filtered solution is divided into several portions which are respectively tested with lime-water ferric chloride, and ferric acetate. These reagents readily dis-

tinguish catechol from pyrogallol in the absence of the other, and will suffice for the recognition of the one in presence of not too large a proportion of the second substance. It must be remembered that the production of pyrogallol may have resulted from the presence of gallic acid in the original substance, if the tannin had not previously been purified therefrom in the manner indicated on page 2. Catechol, on the other hand, may be a product of the decomposition of catechin and other substances allied to and associated with tannins, unless care has been taken to remove them previously. As a general rule, however, catechins and catechol derivatives only occur in quantity with catechol tannins, and the same is true of gallic acid with regard to pyrogallol.

The Stiasny test (*Collegium*, 1911, 318; 1914. 76) may also be applied to ascertain the presence or absence of catechol tannins. It is applied as follows:

50 c.c. of the solution are boiled for 10 minutes with 10 c.c. of 40% formaldehyde and 10 c.c. of hydrochloric acid (1:1), and then cooled and filtered. If the filtrate is tested with 1 or 2 drops of gelatin and salt solution, and 1 c.c. of ferric alum (1%) solution with the addition of 5 grm. of sodium acetate, catechol tannins are completely precipitated by this reagent, pyrogallol tannins giving a blue or violet layer at the junction with the ferric salt. In this way, 5% of myrobalan extract can be detected in quebracho or mimosa extract.

Only catechol tannins form diazo-compounds with diazo-benzene-chloride. This process may be used for estimating the catechol tannins in sumach, the nitrogen being estimated in the precipitate. (Nierenstein and Webster, *Collegium*, 1907, 224.)

Insoluble bromine derivatives are formed from dilute solutions of the catechol tannins by the addition of bromine water. They also generally show a crimson colour with concentrated sulphuric acid, the pyrogallic tannins giving yellow or brown shades.

A. Seyda (*Chem. Zeit.*, 1898, **22**, 1085) describes a delicate test for tannins. He has noticed that if gold chloride is added to a very dilute solution of tannin a clear purple liquid is obtained. The principal use of this test is to determine the presence of tannin in highly coloured extracts. Before being tested they are diluted until practically colourless, the gold chloride is added, and the liquid allowed to stand for half an hour.

W. Vogel and C. Schiller (*Collegium* (1923), 319) recommend nitrosomethylurethane as a reagent. By it catechol tannins are quantitatively precipitated, whereas pyrogallol tannins undergo no change, or only one of colour. From 10–12 c.c. of a 4% tannin solution are boiled with 3–5 drops of 10% HCl; while boiling 5–7 drops of nitrosomethylurethane are added (fume chamber), and the solution is boiled for a further 3–4 minutes, so as to decompose the nitrosomethylurethane. The solution is cooled slowly and filtered. The filtrate is tested for tannins with iron alum and sodium acetate.

The Gold-beater's Skin Test for Tannins.—This is the only specific test known which demonstrates the "tanning" properties of a tannin. It was originally elaborated in the writer's laboratory by Atkinson and Hazleton (*Biochem. J.*, 1922, **16**, 516) and has since been extended in the writer's laboratory by P. H. Price (*Analyst*, 1924, **49**, 25). (For details see qualitative section, p. 78.)

Zellner (*Chemie der höheren Pilze* (1907, 135–137), has shown that frequently different fungi extract different tannins from the same plant. Thus, for example, *Polyporus igniarius* and *P. velutinus* extract a pyrogallol tannin from the oak, whereas *Collybia crassipes* extracts a catechol tannin from the same material. This is of particular interest, since it is claimed by Freudenberg and Vollbrecht (*Ber.*, 1922, **55**, 2420) that oak tannin is a uniform product.

ACTION OF DILUTE ACIDS ON TANNINS. PHLOBAPHENES

As already stated, many tannins are resolved, on heating with dilute acids, into dextrose and either gallic acid, ellagic acid, or an amorphous, insoluble red colouring matter or phlobaphene, according to the nature of the tannin. Other tannins yield these products without dextrose being simultaneously formed. As a rule, the action of dilute acid on a tannin results in the formation, apart from dextrose, of a single decomposition product belonging to the aromatic series (*e. g.*, gallic acid, ellagic acid, phlobaphene, etc.), but in some cases two or more of such substances are obtained.

To ascertain whether a tannin yields dextrose by hydrolysis, it may be freed from any admixture of carbohydrates by precipitation with neutral lead acetate, or saturation of the aqueous solution with salt and removal of the tannin by agitation with ethyl acetate. The washed lead salt, or the tannin left on evaporating the ethyl acetate solution, is then heated to 100° for some hours, with dilute hydro-

chloric acid, in a sealed tube or firmly closed bottle. (Mere boiling with the dilute acid, replacing loss by evaporation, is sufficient in most cases, especially for qualitative purposes.) After cooling and opening the vessel, the mixture should be allowed to stand for some time in the cold, to observe whether any sparingly soluble product separates. (To prevent subsequent error, it is desirable to get rid of any gallic acid, by repeatedly shaking the solution of the tannin with ether, or preferably ethyl acetate, before precipitating with lead acetate.) In such case, the precipitate should be filtered off, and any traces remaining in solution removed by shaking the filtrate first with ethyl acetate and then with ordinary ether. The aqueous liquid is boiled, neutralised with sodium carbonate, precipitated with basic lead acetate (to remove any traces of tannin or colouring matters), the liquid again filtered, the excess of lead removed by dilute sulphuric acid, the filtered liquid again neutralised by sodium carbonate, and heated to the b. p. with Fehling's solution, when a yellow or red precipitate of cuprous oxide will prove the presence of dextrose. This latter may also be indicated by a fermentation test with yeast, or by an optical examination as to rotatory power.

The precipitate obtained on cooling the product of the action of dilute acid on the tannin may consist of lead chloride (if the lead compound has been used), ellagic acid, or a phlobaphene. The lead chloride may be removed by washing with boiling water. If the residue has a pale yellow or fawn colour, and is but slightly soluble in cold alcohol, it probably consists of ellagic acid, which is soluble in ammonia and hot alcohol, and dissolves readily in strong nitric acid giving an intense crimson coloration. (Griessmeyer test for ellagic acid.)

The crude ellagic acid is best crystallised from pyridine (Perkin and Nierenstein, *J. Chem. Soc.*, 1905, **87**, 1417). It is advisable to prepare the tetra-acetyl derivative, which melts at 343–346° (Nierenstein, *Chem. Zeit.*, 1909, 87).

A red-coloured residue, readily soluble in cold alcohol, will consist of a phlobaphene, which will be reprecipitated on diluting the alcoholic solution with water, and may be further examined by fusion with potassium hydroxide or zinc dust distillation.

The *ethereal layer*, obtained by shaking the filtrate from the ellagic acid and phlobaphenes with ether and ethyl acetate, will contain gallic acid, if any has been formed in the treatment of the tannin with

dilute acid. For its recognition, the ethereal solution should be evaporated to dryness, the residue treated with cold water, and the solution filtered. The filtrate will give a fine red colour with potassium cyanide, if gallic acid has been produced. The test may be confirmed by treating another portion of the filtrate with an aqueous solution of picric acid, followed by ammonia, when a reddish colour, changing to a fine green, will be produced if gallic acid be present. In this connection it must be noted that the m. p. of gallic acid cannot be relied on, as gallic acid decomposes into pyrogallol and carbon dioxide *before* it melts.

It is sometimes sufficient to boil the tannin or its infusion with dilute hydrochloric acid for some time, replacing the acid lost by evaporation. The solution is then diluted and allowed to cool, when ellagic acid and phlobaphenes will separate, and may be filtered off and separated by treatment with cold alcohol as already indicated.

Fischer and Freudenberg (*Ber.*, 1912, **45**, 915) have elaborated a method for the hydrolysis of gallotannin. By this method they have shown that Chinese gallotannin gives gallic acid and dextrose on hydrolysis, whereas Turkish gallotannin gives gallic acid, ellagic acid and dextrose by the same method (Fischer and Freudenberg, *Ber.* 1914, **47**, 2485; compare also Karrer, Widmer and Staub, *Annalen*, 1923, **433**, 288). This method has been criticised by Feist and Hahn (*Arch. Pharm.*, 1913, **251**, 468), who find that by Fischer's method much destruction of the dextrose takes place. However, Geake and Nierenstein (*Ber.*, 1914, **47**, 891), Nierenstein, Spiers and Geake (*J. Chem. Soc.*, 1921, **119**, 275), Perkin and Uyeda (*J. Chem. Soc.*, 1922, **121**, 66) and Nierenstein (*Ber.*, 1923, **56**, 1876) have used it with some success.

Fischer and Freudenberg's method has been in constant use in the writer's laboratory since 1912, and in the course of these years it has undergone some slight modifications, with the result that the following procedure is now in use: About 10 grm. of gallotannin are hydrolysed in a boiling water bath for 110 hours with 150 c.c. 10% sulphuric acid. It has been found useful to replace the air condenser, as originally recommended by Fischer, by a Liebig condenser. The gallic acid formed is filtered off, and the filtrate kept on ice for several days, when a second crop of gallic acid is obtained. The filtrate is treated with lead acetate and a little lead carbonate, and the filtered solution freed from lead with hydrogen sulphide. The latter process

has to be carried out in the boiling solution. The filtrate is tested for dextrose. *Fehling's solution is not reliable, as gallic acid by itself yields a product which reduces Fehling's solution on boiling with dilute sulphuric acid.* This is evident from the results obtained by Geake and Nierenstein, (*Ber.*, 1914, **47**, 874) by Bertrand's Volumetric reduction method (*Bull. Soc. Chim.*, 1906, (iii), **35**, 1285) and by the polarimetrical method.

	Dextrose	
	Polarimetrically, %	By reduction, %
Schering's Tann. leviss. pur. I ...	0 91	2 70
	0	4 63
	0.38	7 58
	0	4 60
	0	2 30
	0	5 90
Schering's Tann. leviss. pur. II	5 40	5 28
	5 16	5 68
	5 82	5 51
	4 95	3 75
Kahlbaum's Gerbsäure I	0 27	2 18
	0	3 04
	4 94	5 14
	4 67	6 00
	2 27	4 63
	2 08	4 11
Merck's extra pure, very light, clearly soluble P.B.	0	3 26
	5.01	6 09
	8 58	8 34
	0	2 68
	1.28	4 20
Schuchardt's Tann. lev. pur.	0 10	2 16
	6 75	6 40

As will be seen, the differences between the two methods are striking. Similar results have recently been obtained by Nierenstein (*Ber.*, 1923, **56**, 1876). *It has, therefore, been made a hard and fast rule in the writer's laboratory that only the preparation of the dextrosazone is regarded as real evidence of the presence of glucose.* But even in this case, much care has to be taken, as the melting point of the dextrosazone varies remarkably. It has been found to melt at 205° (Fischer, *Ber.*, 1889, **22**, 365), 206° (Tiemann, *Ber.*, 1886, **19**, 50), 208° (Tollens, *Zeitsch. Vereins Zuckerind.*, 1889, **39**, 917), 210° (Fischer u. Hirschberger, *Ber.*, 1888, **21**, 1805), 213° (Fischer,

Ber., 1908, **41**, 73), and 217° (Tutin, *Proc. Chem. Soc.*, 1908, **23**, 250). The dextrosazone is best crystallised from pyridine as recommended by Tutin, when it melts at 215.5°, and that melting point has been accepted by the writer as its correct melting point. ✓

Phlobaphenes.—The phlobaphenes are probably anhydrides of the respective tannins from which they are derived. They are produced by the action of dilute acids on tannins. There is, however, a marked difference between the naturally occurring "phlobaphenes" and the "phlobaphenes" which are produced by the action of acids. (See Nierenstein and Webster *Collegium*, 1909, p. 337; Manning and Nierenstein, *J. Chem. Soc.*, 1919, **115**, 662.)

They may also be formed in many cases by pouring alcoholic or highly concentrated aqueous solutions of the tannins into cold water, under which circumstances a part of the tannin becomes insoluble, and the phlobaphene separates as a red precipitate. Phlobaphenes exist ready-formed in most tannin materials capable of producing them.

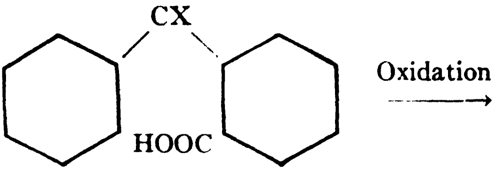
The phlobaphenes are red or brown amorphous substances, difficultly soluble in water, weak acid solutions, or in pure ether, but soluble in water containing ammonia, and freely soluble in alcohol. Some phlobaphenes are so sparingly soluble in water, even when boiling, that this characteristic may be utilised for the estimation of the corresponding tannin. This is especially the case if, after heating with hydrochloric acid, the liquid be evaporated to dryness and the residue treated with water. The decomposition products often remain almost entirely insoluble. The phlobaphenes are also dissolved by dilute alkalies and alkali carbonates, and by borax, which last substance is said to be used in the preparation of some tannin extracts, and has been suggested as a means of rendering phlobaphenes available for tanning. The solubility of the phlobaphenes in water seems to depend on their degree of hydration, many tannins giving a whole series of anhydrides of which those containing one molecule of water less than the original tannin are soluble in water, whilst the higher members of the series become less and less soluble as they lose the elements of water. (Compare Manning and Nierenstein, *J. Chem. Soc.*, 1919, **115**, 662.) The soluble phlobaphenes are the colouring matters of tanning materials, and behave like the tannins themselves, precipitating gelatin and combining with hide to form leather. Hemlock bark yields a series of such substances, of which the lower

members are deep red soluble tannins, and the higher form the red sediment which occurs in hemlock extract. It is not possible to decolorise hemlock extract without at the same time greatly reducing its tanning powers, though by preparing and concentrating it at a low temperature the proportion of insoluble higher anhydrides formed may be kept at a minimum.

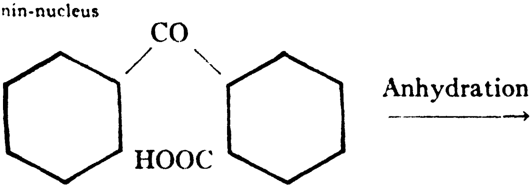
The phlobaphenes somewhat resemble the resins in their properties, as, for example, their solubility in alcohol, and slight solubility in water, and their behaviour when fused with alkali hydroxides; but they are distinguished from the resins by dissolving in dilute ammonia. With gelatin, ferric acetate, and lead acetate the phlobaphenes usually behave like their respective tannic acids. Occasionally a so-called tannin is met with (*e. g.*, hop-tannin), which is not precipitated by gelatin, whilst the phlobaphene produced there-from is precipitated.

These facts have been the object of wild speculation. Thus Moeller (*Zeitsch. Leder Gerb.-Chem.*, 1923, **2**, 343) and also Freudenberg ("*Die Chemie der natürlichen Gerbstoffe*," 1920) suggest that all tannins exist in a crystalline form, when they possess no tanning properties and when they are insoluble in water. On changing into colloids (phlobaphenes) they acquire tanning properties and become soluble in water. That this is not the case is evident from the fact that hamameli tannin, paullinia tannin, and acertannin, all of which are well crystallising substances, give a positive gold-beaters' skin test for tannins.

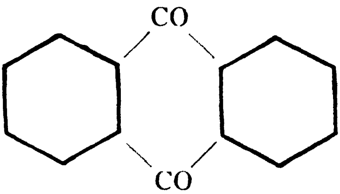
Phlobaphenes are yielded by the tannins from the bark of the oak, elm, horse-chestnut, willow, birch, fir, and acacia, as well as by the tannins from rhubarb, male fern, wine, etc. According to Grabowski, the phlobaphenes from the tannins of the oak, ratanhia, and tormentilla are not merely analogous to, but actually identical with, chestnut-red. According to Nierenstein and Webster (*Ber.*, 1908, **41**, 80; Collegium, 1909, p. 378; also Nierenstein, *Ber.*, 1909, **42**, 353) phlobaphenes yield diphenylmethane and anthracene on distillation with zinc dust. These reactions are interpreted by them as follows:



Tannin-nucleus



Nucleus of α-phlobaphene
(yields diphenylmethane)



Nucleus of β-phlobaphene
(yields anthracene)

ACTION OF FUSED ALKALI ON TANNINS

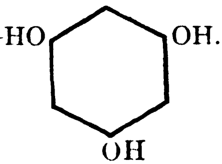
When tannins are subjected to the action of alkali hydroxide in a state of incipient fusion, they are decomposed, with formation of products varying with their constitution. Tannins yielding catechol on dry distillation, that is, all those which give a green colour with ferric acetate (and valonia and oak-bark tannins, in addition) give protocatechuic acid when fused with potassium hydroxide. On the other hand, those tannins which give pyrogallol when heated alone yield gallic or ellagic acid when fused with alkali hydroxides. In each case the action brings about the elimination of CO_2.

The tannins which yield protocatechuic acid on fusion with alkali hydroxides may be further subdivided according to the secondary product formed simultaneously, one class giving acetic or some other fatty acid, and a second class, phloroglucinol. A third class, including the tannins of the alder and hop, give both acetic acid and phloroglucinol, but this peculiarity is not improbably due to the co-existence of 2 distinct tannins. The fusion of gallic acid with sodium hydroxide is said to result in the formation of a small quantity of phloroglucinol (*J. Chem. Soc.*, 1883, **44**, 60).

To recognise the presence of a phloroglucinol tannin without employing the tedious method described below, Procter mixes 5 c.c. of water, 1 c.c. of a saturated solution of commercial aniline nitrate, and 1 c.c. of a very dilute solution of potassium nitrite. To this liquid is added 1 c.c. of a solution containing as nearly as possible 0.5 per cent. of the tannin to be examined. If phloroglucinol or phloroglucinol tannin be present, the liquid will gradually become yellow or orange, and will deposit a cinnabar-red precipitate after standing for a time not exceeding 1 hour, but many other substances also give a precipitate under these conditions. Thus the indication is produced by oak-bark infusion, which is not supposed to contain a phloroglucinol tannin, and gall tannin, pyrogallol, and other substances give similar but browner precipitates. A sharper distinction may be obtained by employing dilute solutions, but it is advisable also, whenever possible, to act on the tannin with fusing potassium hydroxide and examine the products.

The fusion with potassium hydroxide may be conducted on the original tannin, or on the substance produced by treating it with dilute acid. The lead salt may be substituted for the free tannic acid. The separation or recognition of protocatechuic and gallic acids or pyrogallol when mixed is troublesome, and hence it is more satisfactory in most cases to aim at the isolation and recognition of phloroglucinol. The following method may be followed: 20 grm. of the tannin, phlobaphene, or lead salt are boiled with 150 c.c. of a solution of potassium hydroxide of 1.2 sp. gr. for 2 or 3 hours, and the liquid then concentrated, with continued stirring until it becomes pasty, the mixture then undergoing fusion. In some cases, such as that of phloretin, it is sufficient to boil the substance with potassium hydroxide solution, as described, omitting the subsequent evaporation, and fusion. The product is cooled, and treated with dilute sulphuric acid in quantity sufficient to render the whole distinctly acid when cold, the liquid is filtered from the potassium sulphate and other solid matters, and the filtrate is treated with sodium hydrogen carbonate till its wine-red coloration with litmus (or absence of red coloration with methyl-orange) shows that the sulphuric acid is neutralised. The liquid is then shaken several times with ether, and the ethereal solution evaporated. The residue contains phloroglucinol, recognisable by its sweet taste and its reactions with ferric chloride and pine-wood. If necessary, it may

be purified from protocatechuic acid by dissolving it in ether and shaking the ethereal solution with dilute sodium bicarbonate saturated with CO_2, which only dissolves the protocatechuic acid. On evaporation of the ether phloroglucinol is obtained. The bicarbonate solution is acidified with dilute sulphuric acid and extracted with ether, which leaves the protocatechuic acid on evaporation. *All ethereal extracts have to be washed carefully so as to free them from acid.*

Phloroglucinol. Phloroglucin.— 

This substance is isomeric with pyrogallol and hydroxyquinol. It possesses both hydroxylic and ketonic properties. The ketonic form yields a *trioxime*, which turns black at 140° and explodes at 155°. The phenolic form yields a *triacetate* (m. p. 104–106°) and a *trimethylate* (m. p. 52.5°). Phloroglucinol forms small plates or rhombic tablets containing $2H_2O$. It becomes anhydrous at 100°, and melts at 219–220° if heated rapidly, but at 209° or even 200° if slowly heated. At a higher temperature it sublimes without odour, and solidifies again on cooling.

Phloroglucinol is sweeter than cane-sugar. It is soluble in water and alcohol (1 : 100), and readily in ether, and by agitation with the last solvent can be removed from its aqueous solution. The following specific tests are given for phloroglucin:

(1) Ferric chloride: blue-violet coloration.

(2) The aqueous solution gives by the addition of bromine water a precipitate of $C_6H_3Br_3O_3$ (Tribromo-phloroglucinol).

(3) Silver nitrate produces a precipitate in the cold solution on standing. Addition of ammonia and boiling gives reduction.

(4) *Millon's*-reagent: No coloration.

(5) *Guareschi-Lustgarten* test: Negative.

(6) *Liebermann* reaction: blood-red coloration, turns violet on dilution with water.

(7) *Berthelot-Lex* test: Ammonia faint violet coloration, turns yellow on the addition of hypochlorite. Warming produces a red coloration.

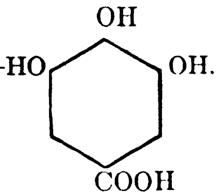
(8) *Vanillin and hydrochloric acid* test: red or red-violet.

(9) *Formaldehyde and sulphuric acid* test: faint red ring, turns reddish-yellow on shaking.

(10) The aqueous solution turns blue-violet on the addition of sodium hydroxide and shaking. Addition of hydrogen peroxide gives the reaction better than shaking. (Main difference from pyrogallol, which turns yellow.)

(11) If a freshly cut slip of pine-wood is moistened with a dilute solution of phloroglucinol, and subsequently with hydrochloric acid it acquires an intense violet or red colour.

Gallic Acid.—



This substance crystallises from water in small needles, which contain 1 mol. of H_2O and loses its water of crystallisation at 120°. It melts at 239–240° when carefully heated, carbon dioxide being evolved. This melting point is not reliable, and it is often found to melt as low as 200°, owing to the formation of pyrogallol: $C_6H_2(OH)_3.COOH = C_6H_3(OH)_3 + CO_2$. Gallic acid is soluble in water (1:130 at 12.5°; 1:3 at 100°) and in alcohol (1:4.5). It is also soluble in acetone, ether, ethyl acetate and hot chloroform.

Special Tests for Gallic Acid.—(1) Reduces Fehling solution in the cold.

(2) Ferric chloride gives a blue-black coloration.

(3) On heating with concentrated sulphuric acid at 140° a deep-red coloration (sometimes deep red precipitate) of rufigallic acid is formed.

(4) According to Nasse (*Ber.*, 1884, **17**, 1166) iodine in the presence of neutral salts produces a purple-red coloration. The same coloration is also given by pyrogallol and gallotannin.

(5) The *Young* Test.—The addition of potassium cyanide produces a red coloration, which disappears on shaking. According to observations made by the writer this test is not given by: gallotannin freed by his method from gallic acid, methyl- and ethyl-gallate, m-digallic acid from gallotannin and pyrogallol.

(6) The *Böttinger* Test.—The original Böttinger test (*Annalen*, 1890, **256**, 341) has been modified in the writer's laboratory as follows: To a dilute solution of 0.5 grm. gallic acid in 50 c.c. of water are added a few drops of a 1% solution of phenylhydrazine in acetic acid. The solution is heated in a boiling water bath for 30 minutes and then made alkaline with sodium hydroxide, when a yellow coloration is produced.

(7) The *Berthelot-Lex* Test.—To a solution of gallic acid are added ammonia and a few drops of hypochlorite. A deep green coloration is produced on standing, which turns yellow on warming.

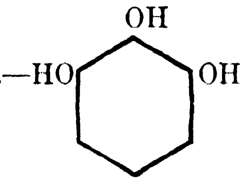
(8) The *Guareschi-Lustgarten* Test: Negative.

(9) The *Millon's* Test: Negative, even on warming.

(10) The *Formaldehyde* and *sulphuric acid* Test: Red ring, the solution turns red on shaking.

(11) *Vanillin-Hydrochloric Acid* Test: Negative, even on warming.

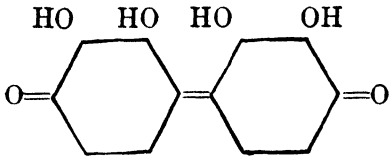
Pyrogallol.—

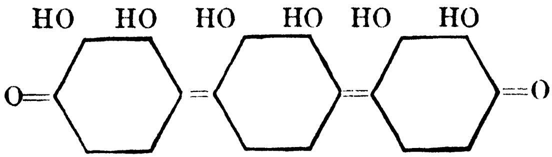


A frequent by-product when gallic acid is obtained, especially on hydrolysis with alkali. It crystallises in thin leaves or needles. M. p. 132–133°. It sublimes without decomposition. It is soluble in water, (1: 2.25 at 13°; 5: 1 at 100°), alcohol (1: 2.25 at 15°), and ether (1: 1.27 at 12°); and also to some extent soluble in chloroform, benzene, and other organic solvents. It gives a characteristic phenylurethane (m. p. 178°).

Special Tests for Pyrogallol.—(1) The aqueous solution turns yellow on the addition of dilute alkali during which process 2, 3, 2′, 3′—tetrahydroxy—4, 4′—diphenoquinone (I) and 2, 3, 2′, 3′, 2″, 3″—hexahydroxy—4, 4″—triphenoquinone (II) amongst other products are produced (Harris, *Ber.*, 1902, **35**, 2954; Nierenstein and Rixon *Annalen.*, 1912, **394**, 249 Nierenstein, *J. Chem. Soc.*, 1915, **107**, 1218).

I





II

(2) Reduces Fehling solution in the cold.

(3) According to Taquemin (*Bull. Soc. Chim.*, 1874, **21,** 222) and Cazeneuve and Linossier (*Bull. Soc. Chim.*, 1885, **44,** 114) pure ferrosulphate produces a white turbidity, which turns blue, then brown-red on the addition of sodium hydroxide.

(4) Ferric chloride gives a red coloration, which turns blue on the addition of calcium carbonate.

(5) Ammonium molybdate: red-brown coloration.

(6) The *Millon's* Test: yellow on warming.

(7) *Guareschi-Lustgarten* Test: Negative.

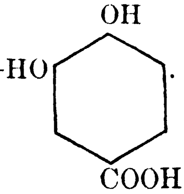
(8) The *Berthelot-Lex* Test: The cold solution turns dark violet and becomes brown on warming.

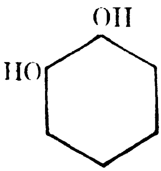
(9) The *Vanillin and Hydrochloric* Acid Test: Faintly-reddish colour in the cold, which turns deeper red on warming.

(10) *Formaldehyde and Sulphuric Acid* Test: Red ring, the solution turns red on shaking and becomes cloudy.

COMPARISON BETWEEN GALLIC ACID AND PYROGALLOL

	Gallic acid	Pyrogallol
Formaldehyde + HCl	Red on warming	Red in the cold
Lime-water	Permanent blue coloration	Faint, possessing blue coloration
Sodium hydroxide	Brown-red	Brown
Vanillin + HCl	Negative	Red
Millon's reagent	Yellow	Red
Berthelot-Lex test (in the cold)	Green	Violet
Heating with conc. H_2SO_4	Red	Brown-red

Protocatechuic Acid.—. This substance is obtained as small needles from water. It crystallises with 1 mol. H_2O which is lost at 105°. The melting point is given as 194–195° and 199°. From experiments carried out in the writer's laboratory it has been found that protocatechuic acid melts at 195°, when slowly heated, carbon dioxide being evolved. It is soluble in water (1:50 at 14°, and 1:16 at 100°), easily soluble in warm alcohol, ether, and practically insoluble in benzene (by which protocatechuic acid can be separated from other hydroxy-benzoic acids). Ferric chloride produces a deep green coloration, which turns dark red on the addition of sodium bicarbonate.

Catechol. . It crystallises from water in needles, which melt at 104° and boil at 245°, without decomposition. If crystallised from benzene leaflets are obtained, whereas petroleum spirit and ether give small prismatic scales. (Compare Beckenkamp *Zeitsch. f. Krystallographie*, 1900, **33**, 599; also Negri: *Gazz. Chim. Ital.*, 1896, **26**, I, 75.)

Special Tests for Catechol.—(1) Ferric chloride gives a deep green coloration, which turns red on the addition of sodium carbonate, and violet on the addition of sodium acetate. (Compare Müller, *Zeitsch. anal. Chem.*, 1876, **15**, 465.)

(2) Bromine water gives no precipitate, whereas phloroglucinol gives tribromo-phloroglucinol.

(3) The *Millon's* Test.—Turns brown-red instantaneously.

(4) The *Liebermann* Test.—Dark brown, turns blood-red on dilution with water, and addition of ammonia.

(5) The *Guareschi-Lustgarten* Test.—Negative.

(6) The *Berthelot-Lex* Test.—Greenish coloration in the cold.

(7) *Vanillin and Hydrochloric* Acid Test.—Red.

(8) *Formaldehyde and Sulphuric* Acid Test.—Violet ring; on shaking, dirty violet coloration.

A. G. Perkin (*J. Chem. Soc.*, 1896, **62**, 1289, 1299, 1303; 1897, **71**, 1131, 1194; 1898, **73**, 374, 1016; 1900, **77**, 423), who has studied the action of alkalies on the tannins and on the yellow coloring matters which are present at the same time in the plant, has come to the conclusion that there is a close relationship between these two groups. This is evident from the following summary: (Perkin, *J. Chem. Soc.*, 1897, **71**, 1338).

	Tannin	Decomposition products of tannin	Colouring matter	Decomposition products of colouring matter
Quebracho colorado	Quebracho-tannic acid	Phloroglucinol	Fisetin	Resorcinol and protocatechuic acid
Rhus coriaria	Gallotannic acid	Gallic acid	Myricetin	Phloroglucinol and gallic acid
Rhus cotinus				
Gambier catechu	Catechin	Phloroglucinol and protocatechuic acid	Quercetin	Phloroglucinol and protocatechuic acid.
Acacia catechu	Catechin		Quercetin	
Colpoon compressum	A catechol tannin	Protocatechuic acid	Quercetin	Phloroglucinol and protocatechuic acid
Divi-divi, &c.	Ellagitannic acid	..	Ellagic acid	..

As will be seen, quebracho tannin is the only exception, since it gives phloroglucinol and protocatechuic acid, whereas the colouring matter yields resorcinol and protocatechuic acid. Nierenstein, however, (*Collegium*, 1905, 70; *Ber.* 1924, **57**, 356) has shown that quebracho tannin contains the resorcinol nucleus, and this has been confirmed by Einbeck and Jablonski: (*Ber.*, 1923, **56**, 1906).

Action of Other Reagents on Tannin.—By heating a mixture of gallotannin, potassium hydrogen sulphate and ethyl acetoacetate at 190–200°, two different compounds are obtained: I. *Ditannacetoacetic ester*, a yellowish-grey powder which is slightly soluble in cold water and decomposed by hot water; soluble in alcohol and acetic ether. 2. *Tannacetoacetic ester*, which is scarcely soluble in either cold or hot water, readily soluble in alcohol, ether, and ethyl acetoacetate. By the action of glycerin on a mixture of gallotannin and potassium hydrogen sulphate, substances are produced which, according to their empirical formulae, are reduced anhydro-derivatives of gallotannin or gallic acid, with properties similar to hydroquercitannic and hydroquergallic acids, the reduction products of

the oak-bark tannic acids. From the mixture so obtained have been isolated: (1) *Hydrotannin* $C_{14}H_{14}O_7$, possessing the properties of tannin, but with stronger reducing power. It is a brown powder, soluble in ammonia, alcohol, and dilute acetic acid, but insoluble in water. (2) *Isohydrotannin*, $C_{14}H_{14}O_7.H_2O$, a brown powder, soluble in ammonia and dilute boiling alcohol, and slightly so in hot water but insoluble in cold water.

If gallotannin is heated with glycerin or dextrose, a compound is formed which is readily soluble in water and dilute acetic acid. The *tannin glyceride* is obtained as a colourless or slightly brownish-coloured syrup, whilst the *glucoside* is a solid substance which forms a syrup with water.

P. Sisley (*Rev. Gen. Mat. Col.*, 1897, **16**, 219) gives a resumé of the effect of various agents on tannin substances. Tannin solutions when exposed to the air absorb oxygen and become brown in colour. A series of oxidation products is obtained, acid in character, and rendering tannin solutions unfit for mordanting in light shades. This oxidation does not seem to be dependent upon fermentation, and takes place more rapidly in dilute than in concentrated extracts. The presence of acid retards the change, whereas alkalies accelerate it in consequence of the phenolic character of tannin. Reducing agents bring about a decolorisation of dilute tannin solutions, which, however, is not permanent, the reducing agent itself becoming oxidised. The protosalts of metals which are capable of different degrees of oxidation and which act as oxygen carriers behave similarly. With concentrated extracts sulphurous acid is useful and preserves them comparatively well, but with diluted extracts the sulphurous acid is oxidised too rapidly. Arsenious and phosphorous acids, however, give satisfactory results when used in small quantities.

In a later investigation it was shown by Sisley (*Bull. Soc. Chim.*, (IV), 1909, **5**, 727) that gallotannin yields ellagic acid when its alkaline solutions are exposed to the air. Similar observations had previously been made by Buchner (*Annalen*, 1845, **52**, 363), Schiff (*Annalen*, 1873, **170**, 79; *Ber.*, 1879, **12**, 1534), and Herzig and Broneck (*Monatsh.*, 1908, **29**, 248). This has since been confirmed by Trunkel (*Arch. Pharm.*, 1911, **248**, 202) and by Nierenstein, Spiers and Geake (*J. Chem. Soc.*, 1921, **119**, 275). The latter have shown that gallotannin yields 60% of ellagic acid under these conditions.

Dilute acids hydrolyse tannins at high temperatures, with the formation of gallic acid. Gallotannin is also hydrolysed by hydrogen sulphide, gallic acid being produced.

Solutions of tannins usually contain varying quantities of nitrogenous and pectic substances, glucosides, and mineral constituents, and these facilitate the growth of fungi and yeasts. This fermentation converts the tannin into gallic acid and dextrose: further action then takes place, carbonic, butyric, oxalic, and lactic acids being formed. The glucosides present also undergo alcoholic fermentation and give rise to the wine-like odour to be noticed in fermented extracts.

The conversion of gallotannin into gallic acid with the aid of different kinds of *Aspergillus* and *Penicillium* has been the object of several patents (compare for example Calmette, D. R. P., 1900, 129164) and much research. It has been shown by Fernbach (*Compt. rend.*, 1910, **131**, 1214) and Pottevin (*Compt. rend.*, 1900, **131**, 1215) that this is due to the enzyme *tannase*. Tannase has since been prepared by different workers (Dox, *Bull.* No. 120, U. S. Dept. of Agriculture, 1910; Knudson, *J. Biochem.*, 1913, **14**, 159; Freudenberg, *Ber.*, 1920, **52**, 177; Rhind and Smith, *Biochem. J.*, 1922, **16**, 1). Methods to measure the activity of tannase have been elaborated by Rhind and Smith (*loc. cit.*) and Freudenberg and Vollbrecht (*Zeitsch. physiol. Chem.*, 1921, **116**, 277). This latter method has been investigated in the writer's laboratory by D. Rhind who has found it to be unreliable (*Analyst*, 1924, **49**, 505.) In a series of investigations which have been in progress in the writer's laboratory it was found by Miss W. N. Nicholson that a slight modification of Mitchell's colorimetric method (Vol. III, p. 566) gives the best results. Miss Nicholson's modification of this method is given on page 187.

Gallotannin.—Tannic Acid. Tannin.

Gallotannin occurs in gall-nuts in proportions commonly ranging from 60 to 77%, and is usually prepared therefrom by the method of Pelouze described on page 2.

Another plan is to extract gall-nuts with a mixture of 12 parts of ether and 3 of alcohol, 12 parts of water being added to the extract, and the alcohol and ether removed by distillation. The residual aqueous solution is then filtered and evaporated, the product being further purified by solution in water and treatment with animal

charcoal. To obtain the tannin in a spongy form, the syrup solution should be mixed with alcohol and ether and evaporated at a moderate temperature. The spongy form dissolves very readily.

Gallotannin may also be obtained, according to Schiff, by extracting gall-nuts with anhydrous ether to which 5% of alcohol has been added.

As prepared by Pelouze's process, tannin yields more or less dextrose or an analogous substance when treated with dilute acids (the amount obtained varying from o to 22%), gallic acid being formed at the same time. Ordinary tannin has been represented by the empirical formula $C_{34}H_{28}O_{22}$, which would yield 23% of dextrose on hydrolysis. However, recent investigations have shown it to contain from 8–12% dextrose only. As prepared by Schiff's process, gallotannin yields little or no dextrose on treatment with dilute acid, though agreeing in its other characters with the product obtained by Pelouze's method.

This question has in recent years been the object of much discussion. Whereas Emil Fischer maintains that gallotannin is a derivative of glucose, it has recently been found by Mitchell (*Analyst*, 1923, **48**, 2) that a specimen of gallotannin examined by him contained little or no glucose. Mitchell's gallotannin has been re-examined by Nierenstein (*Analyst*, 1923, **48**, 321; *Ber.*, 1923, **56**, 1876), who has confirmed Mitchell's results.

H. Schiff claims to have obtained a gallotannin synthetically by taking gallic acid dried at 110°, mixing it into a thin paste with phosphorus oxychloride, and heating the mixture first to 100° and then at 120°. Hydrogen chloride is evolved, and the gallic acid is converted into a yellow powder, which should be washed with ether and dissolved in water. The unchanged gallic acid is allowed to crystallise out, after which the solution is saturated with common salt, the precipitated tannin is washed with brine and redissolved in ether-alcohol. The product thus obtained gives all the reactions of purified gallotannin, but is perfectly reconverted into gallic acid on boiling with hydrochloric acid. Schiff's experiments have so far not been confirmed, although they have also not been disproved. (Compare, Biginelli, *Gaz. chim. ital.* 1909, **39**, II, 268, 283.)

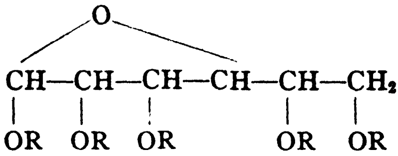
It has been generally considered that pure gallotannin or tannin from galls has the composition $C_{14}H_{10}O_9$, and in constitution is the anhydride of gallic acid, or *digallic acid*. It is still suggested

in certain quarters that tannin is a glucoside. Feist in 1908 suggested a formula, $C_{27}H_{22}O_{17}$, based on this assumption. Nierenstein and Sisley hold the opposite view, contending that those investigators who still consider that tannin is a glucoside, have not removed all the tannin and gallic acid from the solution before examining its reducing power with Fehling's solution.

However, the classical investigations of Emil Fischer have disproved this view, as he has conclusively shown that Chinese and Turkish gallotannin give dextrose on hydrolysis. In this connection reference must, however, be made to Mitchell's observation that gallotannin may contain little or no dextrose. *These differences are due to the one fact only that no method has so far been devised by which a pure gallotannin can be prepared.* (Compare, Nierenstein, *Analyst*, 1925, **50**, 604.) This is particularly evident from the investigation of Iljin (*Ber.*, 1914, **47**, 895) who has shown that gallotannin having $[\alpha] + 75.0°$ in water gives a series of fractions, where $[\alpha]$ in water is $+137.8°$, $+122.1°$, $+134.3°$, $+106.6°$, $+114.8°$, $+89.1°$, $+21.8°$, $+16.8°$, and this has been confirmed in the writer's laboratory. It has also been shown by Iljin that all these fractions are typical tannins. Iljin's work has been confirmed by Karrer in 1923, and there is not the slightest doubt that gallotannin is not a uniform product. Nierenstein, Spiers and Geake (1921) have summarised the position as follows: Since Scheele's first attempt in 1787 to prepare a pure "Galläpfelsalz" by a method which is, incidentally, similar to that employed by Fischer, the chemistry of gallotannin has been one long chain of disappointments. We find ourselves again faced with the necessity of regarding Fischer's pentadigalloyl-glucose formula and the advance for which it stands with reserve, if not with doubt, and we have consequently to consider the question as still open. In view of these facts, we publish our results without attempting to draw any conclusion as to the constitution of gallotannin. This seems to us the only feasible course at the present stage of the investigation, since we are now engaged in a revision of our previous researches on gallotannin, which were published during 1905–1914.

The following is a brief summary of the two prevailing opinions, but reference must be made to the original papers.

Fischer's results (*Ber.*, 1912, **45**, 915, 2709; 1913, **46**, 1116; 1914, **47**, 2485; 1918, **51**, 1760; 1919, **52**, 829) have led him to the conclusion that Chinese gallotannin is penta-digalloyl-glucose.



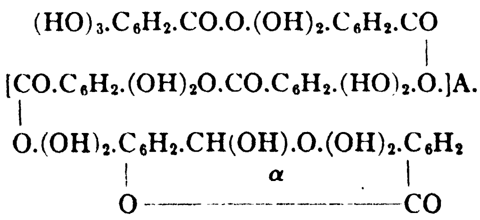
R = Digallic acid radical.

This formula is deduced from the production of gallic acid and dextrose on hydrolysis of Chinese gallotannin and from the *similarity* which exists between synthetic penta-digalloyl-glucose and Chinese gallotannin.

In this connection it must, however, be noted that Fischer's synthetic penta-galloyl-glucose does not give the Goldbeater's Skin test for tannins. It can, therefore, not be regarded as a true tannin (Nierenstein, *Analyst*, 1925, **50**, 604).

Turkish gallotannin differs from Chinese gallotannin, and Fischer has assigned to it provisionally the formula of tetragalloyl-ellagyl-glucose. Investigations which have been carried out by Karrer (*Helv. Chim. Acta*, 1923, **6**, 1; *Annalen*, 1923, **433**, 288) seem to some extent to support these contentions.

Nierenstein's results (*Ber.*, 1905, **38**, 3641; 1907, **40**, 917; 1908, **41**, 77, 3015; 1909, **42**, 1122, 3552; 1910, **43**, 628; *Annalen*, 1911, **386**, 318; 1912, **388**, 223; *Ber.*, 1912, **45**, 1546; 1914, **47**, 891; *J. Chem. Soc.*, 1921, **119**, 275; *J. Amer. Chem. Soc.*, 1925, **47**, 846, 1726), on the other hand, have led to the conclusion that gallotannin is a poly-digalloylleucodigallic acid anhydride:



According to Nierenstein's formula, gallotannin consists of x parts of A. Further, gallotannin may or may not contain dextrose, but, if dextrose is present, it is in combination with the hydroxyl marked α.

Nierenstein's formula has been supported by Mitchell (*Analyst*, 1923, **48**, 2), and recently work published by Nierenstein shows that with the aid of specially cultivated yeast it is possible to free gallo-

tannin from dextrose. *Dextrose is therefore not a normal part of the gallotannin molecule.* Nierenstein has suggested (*Ber.*, 1910, **43**, 1267) that the plant contains, in addition to normal gallotannin, also a glucoside of gallotannin which plays the following rôle in the metabolism of the plant:

$$\begin{array}{ccc} \text{Gallotannin glucoside} & \longrightarrow & \text{Ellagitannin} \\ \downarrow & & \downarrow \\ \text{Gallotannin} & & \text{Luteoic acid} \\ \downarrow & & \downarrow \\ \text{Luteoic acid} & \longrightarrow & \text{Ellagic acid.} \end{array}$$

(Compare also Nierenstein, *Ber.*, 1907, **40**, 4575; 1908, **41**, 3015; 1909, **42**, 353; Drabble and Nierenstein, *Biochem. J.*, 1907, **2**, 96; Nierenstein and Webster, *Ber.*, 1908, **41**, 80; Nierenstein, *Chem. Zeit.*, 1909, **33**, 87; P. Forbes, *Pharm. J.*, 1926, **116**, 225; Nierenstein, *ibid.*, 287.)

"Pure" gallotannic acid forms a colourless amorphous mass, light yellowish buff-coloured scales, or a brittle vitreous mass. It becomes yellow in the light, even if air be excluded. The taste is strongly astringent, and the reaction acid. When heated it darkens, with or without fusing, and at 215° decomposes, with volatilisation of water, pyrogallol, and carbon dioxide, while a residue of *metagallic* or *melanogallic acid*, $C_6H_4O_2$, is left. This last substance is the sole product when tannin is rapidly heated to 280°. It is a black, amorphous, tasteless substance. The optical activity of gallotannin varies, and special reference must be made in this connection to the very interesting investigation by Navassarat. (*Kolloid-chemische Beihefte*, 1914, **5**, 299.)

According to Procter (*Pharm. J. Trans.*, 1889, 351):

100 parts cold water	dissolve	253 parts of dry gallotannin.
100 parts boiling water	dissolve	300 parts of dry gallotannin.
100 parts alcohol	dissolve	120 parts of dry gallotannin.
100 parts chloroform	dissolve	0 007 parts of dry gallotannin.
100 parts benzene	dissolve less than chloroform.	

Gallotannin is precipitated from its concentrated solution by dilute hydrochloric or sulphuric acid, common salt, and potassium chloride and acetate, but not by sodium sulphate or nitric acid. Skin and other gelatinous tissue remove it completely from its aqueous solution. When the solutions are dilute, saline matter must be present to cause precipitation.

In absolutely dry ether, free from alcohol, tannin is almost insoluble; after a certain portion of water has been added the liquid separates into three layers. When 100 grm. of tannin are treated with 150 c.c. of ether, and 100 c.c. of water added, the lowest layer is a concentrated aqueous solution of tannin, the middle layer contains some tannin and much water; whilst the uppermost layer consists of ether holding a little tannic acid in solution.

When taken internally gallotannin is converted into gallic acid, which may afterwards be found in the blood and urine. (Compare Mörner, *Zeitsch. physiol. Chem.*, 1891, **16**, 225; Lewin, *Virchows Archiv.*, 1880, **81**, 74; Stockmann, *Brit. Med. J.*, 1886, II, 1077; Rost, *Archiv f. exper. Pathol. u. Pharmakol.*, 1897, **38**, 346; Neuberg, *Der Harn.* Vol. II, 821.) Tannin diffuses but slowly in aqueous solution, but may be dialysed from its solution in alcohol. It is known that gallotannin is present in a simple form in alcohol, but in a state of molecular aggregation in aqueous solution.

Gallotannin is readily oxidisable. It reduces the salts of gold, silver, mercury and copper, permanganates, etc. Nitric acid rapidly oxidises it, with formation of oxalic acid; and chlorine, bromine, iodine, and chromic acid act readily on it.

Gallotannin decomposes carbonates and acts as a monobasic acid. Its solution in alkali hydroxides rapidly oxidises, and acquires a brown colour. The gallotannates are amorphous and difficult to prepare in a pure state. Most of them are insoluble.

One of the most important and characteristic reactions of gallotannin is the formation of a white (or buff-coloured) flocculent precipitate with a solution of gelatin. This coagulum, which is the basis of leather, is not completely insoluble in pure water, but is wholly insoluble in presence of excess of tannin. When freshly formed it is often extremely finely divided, and passes through the closest filter, but coagulates on adding ammonium chloride, alum, and certain other neutral salts. In this connection it must be noted that methyl-gallate, starch, and vanillin are also precipitated by gelatin. *Since neither of these substances gives a positive goldbeater's skin test for tannins, one must conclude that the gelatin test is not a specific test for tannins.*

Added to a dilute solution of gallotannin, ferrous sulphate occasions no change, if free from ferric salt, but produces a white precipitate in a concentrated solution. With ferric chloride tannin

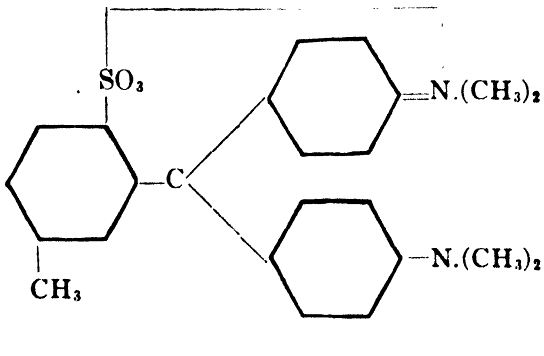
produces a bluish-black precipitate of ferric gallotannate (ink), the colour of which is destroyed by boiling, reducing agents and acids. Ferric chloride detects gallotannin in a solution of 1:30,000. The addition of hydrochloric acid in excess dissolves the precipitate, which is reproduced on adding sodium acetate. Ferric acetate behaves like ferric chloride.

Gallotannin gives no reaction with a solution of cupric sulphate, but on adding excess of ammonia, or in the presence of calcium carbonate, is completely precipitated. The reaction has been used for its estimation. Fehling's solution is reduced by gallotannin on heating.

With tartar emetic, and soluble salts of lead and bismuth, gallotannin yields white insoluble precipitates. With lime-water and with ammoniacal barium chloride it yields a white precipitate, turning blue on exposure.

Sanin, (*Zeitsch. Farbenindustrie,* 1910, **8**, 343) recommends the following reagent for the detection of gallotannin: 20 grm. tartar emetic, 20 grm. sodium chloride, 40 grm. sodium acetate and 5 grm. sodium bitartrate, dissolved in 100 c.c. of water. Sensitiveness; 0.0004 grm. of 80% gallotannin in one litre of water. Gallic acid is not precipitated by Sanin's reagent.

According to Knecht and Hibbert, however, (*New Reduction Methods in Volumetric Analysis,* 1910, 38) Sandmeyer's dyestuff:



of the Patent blue series, *is the only dyestuff which will precipitate gallotannin, but not gallic acid, in the presence of sodium acetate.* This observation has been confirmed in the writer's laboratory with a specimen of Prof. Knecht's dyestuff. The following extract from

their book is of interest: "It is assumed by many that gallic acid is not precipitated by basic colours in the presence of sodium acetate or weak ammonia, and methods for the quantitative estimation of tannic acid have been brought forward which are based on this assumption. As a matter of fact, however, all the ordinary basic colours, *e. g.* magenta, malachite green, safranine, methylene blue, etc., are quantitatively precipitated by gallic acid in the presence of sodium acetate. It is noteworthy that whilst methylene blue yields a pure blue tannate, the gallate is violet."

Gallotannin is not precipitated by calcium acetate from a solution slightly acidified with acetic acid, and the solution remains clear, even after adding twice its volume of alcohol (separation from tartrates, citrates, oxalates, malates, etc.).

With an ammoniacal solution of potassium ferricyanide, gallotannin produces a deep red colour changing to brown, even in very dilute solutions. The test, which was first observed by Allen, is very delicate, but the colour is destroyed by a large excess of the reagent. A somewhat similar behaviour is shown by gallic acid. From observations made in the writer's laboratory it is, however, more or less evident that this reaction is only given by gallotannin specimens which contain free gallic acid.

G. Griggi (*Bull. Chim. Pharm.*, 1899, 5) gives the following reaction: Gallic acid in dilute solution gives a bright ruby-red colour with potassium cyanide, which disappears on standing, but is re-formed on shaking in the presence of air or on the addition of hydrogen peroxide. A solution of tannin or pyrogallol gives a yellowish-red colour with potassium cyanide, which is more slowly decolorised. The addition of an excess of hydrogen peroxide gives a permanent yellow-brown colour with gallic acid, and a dirty white precipitate with tannin.

Ammonium molybdate yields with tannin a red coloration, which is yellow in dilute solutions, and is destroyed on adding oxalic acid.

Löwenthal's permanganate and Mitchell's colorimetric methods may be used for the quantitative estimation of gallotannin.

Commercial gallotannin is often very impure. It may contain more or less dextrose, chlorophyll, volatile oil, gallic and ellagic acids. Starch has been found to the extent of 25%.

Dextrose may be detected by precipitating the solution of the sample with basic acetate of lead, removing the excess of lead with

hydrogen sulphide and heating the filtrate with Fehling's solution. The *glucoside* of gallotannin may be detected by the same method, after boiling the solution with dilute sulphuric acid for ten minutes, and neutralising the solution with alkali.

If *chlorophyll* be present, on shaking the sample with an equal weight of water and the same volume of ether, the ethereal layer will be coloured more or less greenish.

Gallotannin should be entirely soluble in alcohol. If a residue be left, it should be examined for *starch*.

Mineral adulterants will be indicated by ignition. Commerical tannin leaves a very insignificant proportion of ash, 0.4% being apparently the maximum proportion recorded.

Gallic acid may be detected in commercial tannin by separating the tannin with quinine hydrochloride and then testing the filtrate with ferric chloride. Degummed silk absorbs both tannin and gallic acid, but according to Vignon, tannin is much more readily absorbed than gallic acid.

According to observations made in the writer's laboratory by Miss P. H. Price (*Analyst*, 1924, **49**, 25) 0.00005 grm. of gallotannin in 1 c.c. of water is detected with certainty by the gold-beater's skin test for tannins.

A method which is said to be capable of detecting traces of gallic acid in tannin has been described by S. Young (*Chem. News*, 1883, **48**, 31). The sample is dissolved in a little water, ether added equal in volume to about 1/3 of the water used, and the whole well shaken. On standing, 3 layers are formed. The ethereal or uppermost layer is removed and evaporated, and the residue dissolved in water and tested with potassium cyanide, when a strong red coloration will be obtained if the sample contains even a trace of gallic acid. The middle layer contains still more gallic acid, while the lowest aqueous layer is almost free from it. By repeating the agitation with ether several times a more complete separation of the gallic acid can be effected. In this connection it must be noted that the Young test is not given by: *m*- and *p*-digallic acid and methyl-, as well as ethyl-gallate. (Nierenstein, unpublished observations; also *Ber.*, 1910, **93**, 628.)

An estimation of the actual *gallotannin* present in the commercial article may be made by Löwenthal's permanganate method, by the hide powder method, by Nierenstein's caseinogen method, but,

preferably, by Mitchell's colorimetric method. The residue of "non-tannin" does not appear always to consist entirely of gallic acid, dextrose being probably present in some cases. The following percentage results were obtained by T. Maben (*Pharm J.*, (3) 1885, **15**, 852), by applying Löwenthal's method to representative specimens of commercial tannin. *The moisture* was estimated by drying the samples *in vacuo* over sulphuric acid.

	1	2	3	4	5	6	7	8	9
Moisture	5.0	8.0	5.0	7.0	6.0	7.0	3.0	3.0	4.0
Gallotannin	88.8	86.9	54.4	56.9	79.9	77.3	82.3	59.7	70.7
Non-tannin (by difference)	6.2	5.1	40.6	36.1	14.1	15.8	14.3	37.3	25.3
$KMnO_4$ required for "non-tannin"	5.86	1.92	6.01	6.01	1.08	3.08	6.63	5.55	3.89

According to C. Böttinger (*Annalen*, 1888, **246**, 124), even the purest commercial tannin is not a uniform substance, and this is now generally accepted. When heated to 150°, under pressure, with concentrated hydrochloric acid, it gives off a gas burning with a green-edged flame, and on heating the tannin with water and excess of bromine, small quantities of products volatile with steam are produced. Nevertheless such tannin is almost completely fixed by hide, and yields nothing but gallic acid when boiled with aqueous alkali hydroxides. When boiled with a mixture of phenylhydrazine hydrochloride and sodium acetate it becomes intensely yellow, changing to a brownish-yellow coagulated mass on standing. This reaction is said not to take place in the presence of a sugar.

Reference must be made to difficulties which are met with when estimating the moisture of gallotannin (compare Iljin, *Ber.*, 1911, **44**, 3318; Steinkopf and Sargarion, *Ber.*, 1911, **44**, 2904; Fischer and Freundenberg, *Ber.*, 1912, **45**, 915; Geake and Nierenstein, *Ber.*, 1914, **47**, 891).

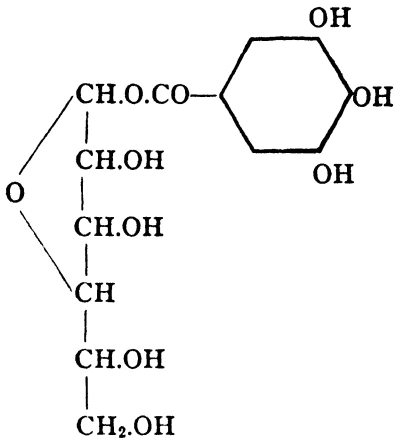
Closely allied to gallotannin are the following tannins, all of which have been obtained in a crystalline form:

Hamamelitannin.—This tannin was originally isolated from *Hamamelis virginica* by Cheney (*Amer. J. Pharm.*, 1886, **4**, 417)

and Grüttner (*Arch. Pharm.*, 1898, **236**, 278), both of whom have obtained it in the form of silky needles, which melt at 115–117°. Grüttner found it to have a rotation of $[\alpha]_D = -35.43°$, in water. He also showed that hamamelitannin is quantitatively hydrolysed into gallic acid on boiling with dilute sulphuric acid. Hamameli tannin has also been studied by Freudenberg (*Ber.*, 1919, **52**, 177; 1920, **53**, 953) who finds, however, $[\alpha]_D = +35°$, in less than 1% solution in water. He has assigned to it the formula $C_{20}H_{20}O_{14}$. According to Freudenberg's observations tannase hydrolyses hamamelitannin, 60% of gallic acid and 34% of an unknown hexose being produced. Freudenberg is of the opinion that hamamelitannin is a digalloyl-hexose. This, however, is not confirmed by Nierenstein who finds that, by the action of a specially cultivated yeast, hamamelitannin can be freed from the sugar when *m*-digallic acid is produced, which thus seems to show that hamamelitannin is probably a glucoside of *m*-digallic acid anhydride. (Nierenstein, unpublished work.)

Chebulinic Acid.—This acid is present in myrobalans (*Terminalia chebula*) and has been obtained in crystalline form by Adolphi (*Arch. Pharm.*, 1892, **230**, 684). Since then it has been the object of several investigations (Thomas, *Arch. Pharm. Inst., Berlin*, 1912, **9**, 78; 1913, **10**, 70; Richter, *ibid.*, 1912, **9**, 85; Fischer and Bergmann, *Ber.*, 1918, **51**, 314; Freudenberg, *Ber.*, 1919, **52**, 1238; Freudenberg and Fick, *Ber.*, 1920, **53**, 1728). Freudenberg has assigned to it the formula $C_{34}H_{30}O_{23}.2H_2O$ (optical rotation in alcohol $[\alpha]_9 + 59 - 67°$). According to Freudenberg chebulinic acid consists of a digalloyl-glucose and one molecule of an unknown acid, having the formula $C_{14}H_{14}O_{11}$. Chebulinic acid has acidic properties and gives a brucine-salt (m. p. 250°). On hydrolysis with tannase gallic acid, glucose and the acid $C_{14}H_{14}O_{11}$ are produced (Freudenberg).

Glucogallin.—This tannin was found by Gilson (*Bull. Acad. Med. Belg.*, 1902, (IV), **16**, 827) in Chinese rhubarb and has been synthesised by Fischer and Bergmann (*Ber.*, 1918, **51**, 1760), who have conclusively shown it to be 1-galloyl-β-glucose:



This is the only tannin with a well-defined chemical constitution. In this connection it must be noted that glucogallin has nothing whatever to do with Feist's glucogallic acid (*Chem. Zeit.*, 1908, **32**, 918; *Ber.*, 1912, **45**, 1493; *Arch. Pharm.*, 1912, **250**, 668; 1913, **251**, 468) isolated by him from Turkish galls, to which Feist has also assigned the same formula. Synthetic glucogallin crystallises from water and melts at 211–213°, whereas the natural product melts at 202–203°. However, both give the same acetyl-derivative, which melts at 125–126° and which has $[\alpha]_D^{18°} = -24.4°$ in acetylene tetrachloride.

Acertannin.—This tannin is present in leaves of *Acer ginnula*, the Korean maple tree (A. G. Perkin, and Uyeda, *J. Chem. Soc.*, 1922, **121**, 66). The crystalline tannin melts at 164–166°. It loses one molecule of water on heating to 125° and a further molecule at 140°. Acertannin crystallises in two forms, viz., prismatic needles $C_{20}H_{20}O_{13}.2H_2O$ and prisms $C_{20}H_{20}O_{13}.4H_2O$. Acertannin is optically active: $[\alpha]_D^{15} = +20{\cdot}55°$ (in acetone). The acetyl derivative melts at 154–155°. It yields, on hydrolysis, gallic acid and a new sugar *aceritol*, which melts at 142–143°. Acertannin is probably digalloylaceritol.

The following amorphous tannins are also closely allied to gallotannin:

Tea Tannin.—Tea tannin has been investigated by Rochleder (*Annalen*, 1849, **63**, 205), Hlasiwetz and Malin (*Zeitsch. f. Chemie*, 1867, **2**, 271), Strauss and Geschwender, (*Zeitsch. angew. Chem.*, 1906, **9**, 1121) and Naninga (*De Bestanddeelen van het Tee-extract*,

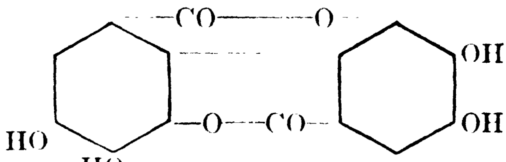
Amsterdam, 1904). The latter has shown it to be optically active: $[\alpha]_D = -177.3°$ (in water) and to yield only gallic acid on hydrolysis with dilute sulphuric acid. Tea tannin from Indian Tea has also been prepared in the writer's laboratory; it was found to agree in every respect with Naninga's data.

Sumach Tannin.—Supposed to be either identical (Löwe, *Zeitsch. anal. Chem.*, 1874, **12**, 128; Gunther, *Russische Pharm. Zeitsch.* 1870, **9**, 161) with gallotannin, or a monomethyl-derivative of gallotannin (Strauss and Geschwender, *Zeitschr. angew, Chem.*, 1906, **29**, 1121.)

Ellagitannin.—This variety of tannin is contained in divi-divi, knoppern and myrobalans, and as a glucoside in pomegranate rind. When boiled with dilute acids or heated with water to 110° in a sealed tube, it loses water and yields ellagic acid. In its other chemical behaviour, ellagitannin closely resembles gallotannin, but gives a light brown precipitate with cupric acetate.

According to Perkin and Nierenstein (*J. Chem. Soc.* 1905, **87**, 1428) ellagitannin is probably galloyl-ellagic acid. Such a compound has been prepared synthetically by Nierenstein (*Ber.* 1911, **44**, 837). Nierenstein (*J. Chem. Soc.*, 1919, **115**, 1174), however, has also shown that the knoppern-gall contains an ellagitannin which is a condensation product of ellagic acid with glucose.

Ellagic Acid.—$C_{14}H_6O_8$,



Ellagic acid was discovered by Braconnot in galls (*Ann. Chim. et Phys.* (2) 1828, **9**, 187) and this was confirmed by Chevreul (*ibid.*, p. 329). The name "ellag," which is the reverse of "galle" was chosen by Braconnot to indicate its presence in galls. Ellagic acid was synthesised by Perkin and Nierenstein (*J. Chem. Soc.*, 1905, **87**, 1413).

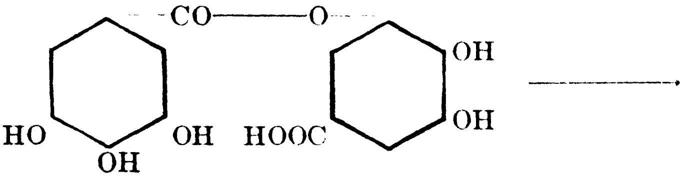
Ellagic acid is formed when a concentrated aqueous solution of gallotannin is exposed for a considerable time to the air, or reacted on with iodine. Ellagic acid is also produced by the dehydra-

tion of ellagitannin (see above), and by the action of oxidising agents on gallic acid. Ellagic acid is readily prepared by pouring a concentrated alcoholic extract of divi-divi into water. The precipitate may be purified by crystallisation from hot alcohol. It may also be obtained by boiling the aqueous extracts of divi-divi, myrobalans, pomegranate rind etc., with dilute hydrochloric acid, and may be purified by solution in alcohol. It may also be prepared by heating gallic acid with dry arsenic acid to 160°, but the product is difficult to purify from arsenic. It may be obtained from bezoar stones (intestinal concretions of a Persian species of goat) by boiling with potassium hydroxide and precipitating with hydrochloric acid. Air-dried ellagic acid contains 1 molecule of water, which it loses at 100° and re-absorbs in moist air. When pure, ellagic acid forms a sulphur-yellow crystalline substance, nearly insoluble in water, even at 100°, and but slightly soluble in alcohol. The aqueous and alcoholic solutions have an acid reaction. It is but slightly soluble in ether, but small quantities may be effectually extracted from the aqueous solution by agitation with that solvent in the presence of sulphuric acid. In potassium hydroxide ellagic acid dissolves to a yellow solution which rapidly becomes darker, and black crystals of potassium *glauco-melanate* separate. Neutral ferric chloride, when shaken with solid ellagic acid is coloured greenish at first, but afterwards becomes inky black. The solution of ellagic acid in hot alcohol has a pale yellow colour, and deposits the acid in sulphur-yellow crystals on cooling. With lead acetate ellagic acid yields a precipitate containing 63% of PbO. Ellagic acid dissolves in fuming nitric acid with a deep crimson coloration. (Griessmeyer test for ellagic acid.)

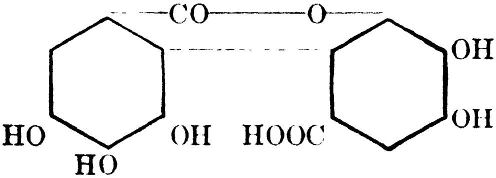
Perkin and Nierenstein give the following details for the preparation of ellagic acid. A solution of 10 grm. of gallic acid in 100 c.c. of boiling acetic acid are treated with 5 c.c. of sulphuric acid, and 10 grm. of finely powdered potassium sulphate are added a little at a time. A somewhat energetic reaction ensues, and from the clear liquid, which rapidly assumes a brown colour, minute, prismatic needles gradually separate. As soon as the ebullition has moderated, the mixture is gently heated for a few minutes, allowed to stand for half an hour, and then poured into water, and the resulting sandy precipitate collected, washed, and dried. It is crystallised from pyridine, from which it separates in needles, which are purified on

re-crystallisation from pyridine with the aid of animal charcoal. The substance thus obtained is an addition compound of ellagic acid with pyridine, which is decomposed on washing with alcohol, when ellagic acid is obtained as a pale yellow powder. Ellagic acid does not melt below 360° and forms a tetra-acetyl-derivative, which melts at 343–346°. This acetyl-derivative has been used by Nierenstein in his comparative studies of ellagic acid from different sources.

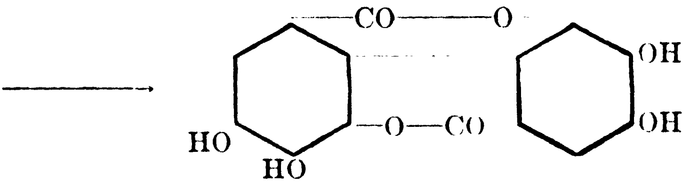
Gallotannin and *m*-digallic acid (Nierenstein, *Ber.*, 1910, **43**, 628; *Annalen*, 1912, **388**, 223) are oxidised on boiling with hydrogen peroxide to luteoic acid and ellagic acid as follows:



m-Digallic acid



Luteoic acid



Ellagic acid.

Luteoic acid crystallises from pyridine in reddish-brown needles, which darken at 305°, and decompose at 338–342°, carbon dioxide being evolved. According to Nierenstein (*J. Chem. Soc.*, 1919, **115**, 1174) knoppern-tannin is a luteoyl-glucose, which on hydrolysis yields ellagic acid as indicated above.

The following tannins have also been investigated but our knowledge of them is still more fragmentary.

Caffetannin.—This variety of tannin occurs in coffee berries. When isolated it forms a brittle mass or a yellowish-white powder. It is only slightly soluble in ether. On boiling caffetannin with dilute sulphuric acid, or by exposing its solution in alkali hydroxide to the air, the liquid acquires a bluish-green colour, owing to the formation of the oxidation product, viridic acid. This substance is characterised by giving a blue precipitate with lead acetate and a crimson colour with strong sulphuric acid. On prolonged boiling with alkali hydroxides, caffetannin yields *caffeic acid*, $C_9H_8O_4$, which crystallises from the neutralised solutions. When fused with potassium hydroxide, caffetannin yields protocatechuic and acetic acids. Heated alone, it gives catechol. Ferric chloride gives a dark green colour with caffetannin, and cinchonine sulphate a white precipitate, but a solution of gelatin is not affected.

According to Goeter (*Arch. Pharm.* 1909, **247**, 184) it consists of chlorogenic acid and some other chromatic substances. Gorter regards chlorogenic acid as the depside of caffeic and quinic acids whereas Freudenberg (*Ber.*, 1920, **53**, 232) on very slender evidence has assigned the formula: $(HO)_2.C_6H_3.CH = CH.CO.O.C_6H_7(OH)_3.COOH$ to it. According to Freudenberg chlorogenic acid is 3.4-dihydroxycinnamoyl-quinic acid.

Quercitannin.—According to C. Etti (*J. Chem. Soc.*, 1883, **44**, 994) the tannin of oak-bark exists in two forms, namely, as quercitannin, and as anhydride of that acid, or phlobaphene.

According to an investigation by Freudenberg and Vollbrecht (*Ber.*, 1922, **55**, 2420) the quercitannin molecule consists of 65 to 67% quercussic acid, 23–25% ellagic acid and 5% dextrose. This seems, however, not to be in agreement with the results obtained by Feist and Schön (*Arch. Pharm.* 1920, **258**, 317), who were not able to detect dextrose in quercitannin.

Feist and Schön's results seem, therefore, to confirm the view that quercitannin is not a glucoside, the reactions which formerly caused confusion being really due to the presence of lævulin, which on treating the oak-bark with dilute sulphuric acid was converted into lævulose.

Quercitannin is amorphous, brownish-red, and readily soluble in water and alcohol. When pure, it dissolves completely in ethyl acetate, but not in pure ether or benzene.

In very dilute alcoholic solution, quercitannin yields a pure yellow precipitate with neutral or basic acetate of lead, but in aqueous solution the precipitate produced is light brown. With ferric salts quercitannin gives a blue-black colour, and yellowish-white precipitates with tartar-emetic, gelatin, albumin, and alkaloids. It is also precipitated by solution of lead nitrate, ammoniacal chlorides of zinc and magnesium, ammoniacal sulphate and acetate of copper, and by molybdate of ammonium. It readily reduces permanganate and Fehling's solution. According to Procter, a dilute solution of quercitannin does not precipitate blood albumin, but renders it uncoagulable by heat, even in presence of free acid.

According to Etti, quercitannin has the composition $C_{17}H_{16}O_9$. At 130° to 140° it gives up water and yields the first anhydride or phlobaphene, $C_{34}H_{30}O_{17}$, which is brownish-red, nearly insoluble in water and in ether, but readily soluble in alcohol or mixtures of alcohol with water. It exists in the original bark, together with quercitannin, and gives a brownish-red precipitate with lead acetate. When boiled with dilute sulphuric or hydrochloric acid, the phlobaphene loses 1 molecule of water and yields a second anhydride, $C_{34}H_{28}O_{16}$, from which a third, $C_{34}H_{26}O_{15}$ may be obtained. All these anhydrides are soluble in alcohol and alkali hydroxides, and are precipitated blue-black by ferric chloride. Löwe has obtained a fourth anhydride, $C_{34}H_{24}O_{14}$, which he designates oak-bark red, a name which has been applied by other observers to the first and second anhydrides.

Tanners class the anhydrides as "colouring matter," and reject barks or extracts containing a large proportion, as they impart too red a colour to the leather, as in the case of red mangrove.

From the number and mode of formation of these anhydrides, together with the evolution of methyl chloride on heating the tannin under pressure with dilute hydrochloric acid, Etti concludes that quercitannin is a methyl-derivative of digallic, or gallyl-gallic acid. Etti also investigated a tannin of the formula $C_{20}H_{20}O_9$, obtained from the bark of a different species of oak. This agreed with the other acid in all its properties, except that it gave a bluish-green colour with ferric chloride, rapidly changing to deep green, and, on addition of sodium carbonate, first to blue and then to red. This variety of tannin yields four anhydrides similar in character to those of the acid with 17 atoms of carbon.

It is possible that the varying statements respecting the composition of oak-bark tannin are due to the presence of 2 analogous substances. According to F. Musset (*Dingl. polyt., J.*, **253**, 8, 340), this is actually the case, both tannins being precipitated by gelatin and oxidised by permanganate. One, which he terms oak-tannin, may be extracted by repeatedly shaking the infusion with acetic ether, in which the oak-red tannin is insoluble. He prefers, however, to estimate the oak-red tannin by precipitation with iodine, avoiding the presence of air. The compound formed contains 7.8% of iodine, and an equal quantity of iodine is converted into hydriodic acid. An equal quantity of the infusion is treated with zinc oxide, and, after 24 hours, and the absence of more than traces of tannin in the filtered solution being proved by gelatin and ferric acetate, the non-tannin matters are titrated with N/10 solution of iodine. By deducting the amount of iodine required by the non-tannin matters from that consumed by an equal measure of the original infusion the iodine which has reacted with the tannins is found, and by subtracting from this twice the quantity of iodine contained in the precipitate of iodine oak-red tannin, the iodine corresponding to the oak tannin is ascertained. Examined in this manner, Musset found German oak-barks to contain 7–8% of oak tannin, and 6–10% of oak-red tannin.

Lupulotannin —The tannin of hops is a glucoside which is easily soluble in water and alcohol, but not in ether. It gives a green colour with ferric salts, a dirty green precipitate with cupric sulphate, a yellow with lead acetate, and a brownish-yellow precipitate with lime-water. It reduces Fehling's solution. Lupulotannin yields a precipitate with albumin but not with gelatin, unless it be previously dried at 100°, by which treatment it is converted into the anhydride or phlobaphene, a substance coexisting with lupulotannin in the hop, and having all the characteristics of a tannin. According to Etti, it is a glucoside which yields protocatechuic acid, phloroglucinol, and dextrose. It precipitates gelatin solution completely, and reduces Fehling's solution. It is soluble in alcohol and in alkalies, and is precipitated on acidifying the latter solution.

Hemlock Tannin.—The tannin of hemlock was investigated by Böttinger (*Ber.*, 1884, **17**, 1041) who has assigned to it formula $C_{20}H_{18}O_{10}$, on the basis of the bromo-derivative $C_{20}H_{14}O_{10}Br_4$, obtained by him by the action of bromine on the aqueous extract of the bark.

Manning and Nierenstein (J. Chem. Soc., 1919, **115**, 662) have, however, shown that Böttinger's bromo-hemlock-tannin's, which contains 43.60% bromine may be resolved into a series of bromo-derivatives which contain from 40 to 49% bromine. Böttinger's bromo-derivative is therefore a mixture. For further work on hemlock tannin see Manning and Nierenstein (*loc. cit.*)

Quebracho Tannin.—The tannin of quebracho-wood has been investigated and has been prepared by Azata (*Ann. Soc. Cient. Argent.*, 1878, **7**, 247), Nierenstein (*Collegium*, 1905, 69; *ibid.*, 1906, 141; *Ber.*, 1907, **46**, 4575), Körner and Petermann (*Zeitsch. angew. chem.*, 1906, **19**, 206) and Strauss and Geschwender (*ibid.*, 1121). The formula $C_{43}H_{50}O_{20}$ has been suggested for quebracho tannin. (Strauss and Geschwender.)

Catechu-tannin, Mimotannin.—The tannins which yield catechol when heated, differ from the pyrogallol derivatives by giving a green coloration with ferric acetate. Like oak-bark tannin, they give insoluble red phlobaphenes or anhydrides by the action of dilute acids. Their constitution is in most cases imperfectly understood. The tannin of catechu is typical of this class of products.

Catechu-tannin, probably identical with the substance described as mimotannin, is the astringent substance contained in catechu (cutch) and gambier. It is extracted by cold water from catechu, and is also formed by heating catechin alone to 130°, with water to 110°, or by boiling it with alkali hydroxides. Catechu-tannin is a dark reddish-brown powder, moderately soluble in water, insoluble in ether, but readily soluble in alcohol and in ethyl acetate. It resembles gallotannin in many of its characters, but gives a greyish-green precipitate with ferric salts, and no reaction with ferrous salts. It is also distinguished from gallotannin by giving a dense precipitate with cupric sulphate and none with tartar emetic, and by yielding catechol and phloroglucinol by fusion with potassium hydroxide. The aqueous solution is precipitated by gelatin, albumin, and dilute sulphuric acid. When treated with hydrochloric acid and potassium chlorate in excess, catechu-tannin yields a chlorinated-substitution-product which is turned purple-red by sodium sulphite. Catechin gives the same reaction.

In view of the above mentioned observations, it is assumed by Freudenberg (*Ber.*, 1920, **53**, 1417) that the catechu-tannins are derived from catechin. This is not confirmed by Nierenstein's

observation (*J. Chem. Soc.*, 1922, **121**, 23) that *Paullinia sorbilis* contains a crystallising tannin—paullinia-tannin—to which Nierenstein has assigned the formula

$$\begin{array}{c} \qquad\qquad O.C_6H_{11}O_5 \\ \qquad\qquad | \\ C_{15}H_8O(OH)_5.CO.O.C_{15}H_8O(OH)_3.COOH. \end{array}$$

This tannin is a true glucoside (not an acyl-glucose, the general formula given by Fischer to the tannins). It yields on hydrolysis gambier-catechin-carboxylic acid and dextrose. It is further shown by Nierenstein that gambier-catechin-carboxylic acid easily loses carbon dioxide when gambier-catechin is produced:

$$C_{15}H_8O(OH)_5.COOH = C_{15}H_9(OH)_5 + CO_2$$

Nierenstein is therefore of the opinion that the catechins are probably derived from the catechu-tannins, and not that the catechu-tannins are derived from the catechins, as supposed by Freudenberg. It is possible that there are two kinds of catechu-tannins, those normally present in the plant, and which yield catechin, and pseudo-tannins which are derived from catechin on boiling the aqueous solution of catechin, which is evident from the fact that catechin gives on boiling a product which precipitates gelatin (Nierenstein, *Analyst*, 1923, **48**, 542). This probably accounts also for the statements made by Freudenberg (*Ber.*, 1920, **53**, 236; 1922, **55**, 1734, 1940) that catechin itself precipitates gelatin. This is contrary to our knowledge of catechin (Compare, Procter, "Leather Industries Laboratory Book, p. 139 [1908], who states that: "unlike tannins, it does not precipitate gelatin.") It is also contrary to Nierenstein's observations (*J. Chem. Soc.*, 1922, **121**, 26; *Ber.*, 1922, **55**, 3832).

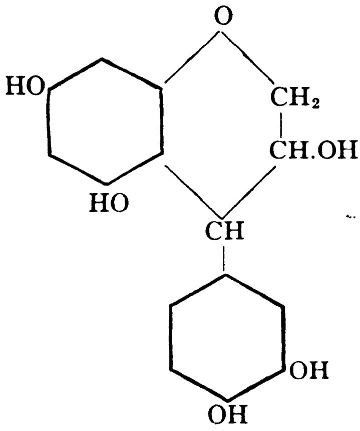
THE CATECHINS

Indian cutches (the extracts of the heart-wood of *Acacia catechu* Willd., A. *catechuoides* Benth, and A. *sundra* D. C.) and cube gambier (the extracts of the leaves of *Uncaria gambier*, Roxb., U. *acida* Roxb. U. *dasyoneura* Korth, *U. Benaysii* Null. and U. *lanosa* Woll) contain from 6–40% of "catechin." "Catechin" has also been found in mahogonywood, in Chinese rhubarb, guarana-paste (the extract of *Paullinia sorbilis* H. Bth. & Kth.), quebracho-wood, cacao-beans and in several Australian Kinos (the extracts of *Eucalyptus vivimatis* Lab., *E. Leucoxylon*, F. Null. and *Angophora intermedia* D. C.).

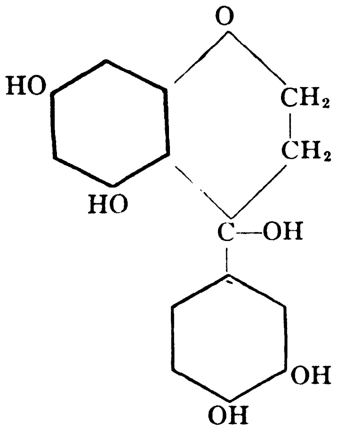
The "catechins" from these sources differ in many respects, and "catechin" is obviously not a single substance, but a series of allied substances. It is, therefore, to be regretted that most workers who are closely connected with the chemistry of "catechin" omit to state the source of the catechin they have investigated.

The constitution of acacatechin—one of the catechins present in cutch—has been established by Nierenstein (*J. Chem. Soc.*, 1920, **117**, 971, 1157, 1594; 1921, **119**, 164; 1922, **121**, 604; *Ber.*, 1922, **55**, 3831; 1923, **56**, 1877; *J. Amer. Chem. Soc.*, 1924, **56**, 2100, 2793, 2798), who has synthesized acacatechin and isoacacatechin. However, these results are in disagreement with those obtained by Freudenberg (*Ber.*, 1920, **53**, 1416; (1921), **54**, 1204; 1922, **55**, 1941; 1923, **56**, 1190, 2127; *Annalen*, 1924, **436**, 286; **437**, 274) and reference must be made to the original papers.

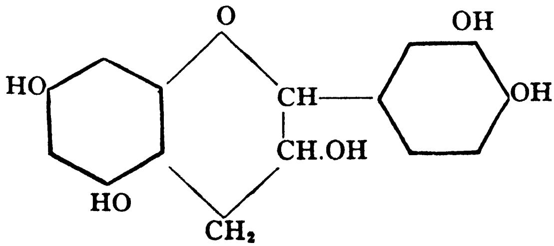
The formulae given by Nierenstein to acacatechin and isoacacatechin are shown in formulae I and II respectively. Freudenberg's formula for catechin regards iso-acacatechin as a stereoisomeric catechin (epicatechin) is shown in formula III.



I



II



III

The catechins are well-crystallising substances, and catechins melting at: 165°, 175°, 204–205°, 212°, 226–228°, 228–230°, 235–237° have been obtained in the writer's laboratory.

The aqueous solutions of catechins give white precipitates with lead acetate and mercuric chloride, but, unlike tannins, they are not precipitated by alkaloids, tartar-emetic or gelatin (compare, Nierenstein, *Analyst*, 1923, **45**, 542). According to an investigation carried out by D. Hooper, in the writer's laboratory, Chapman's cinchonine method for the estimation of tannins, (*J. Inst. Brewing*, 1907, **13**, 646; 1909, **15**, 360) can be used for the estimation of tannins in the presence of catechins, as the latter are not precipitated by cinchonine.

Hooper also finds that hide-powder absorbs catechin. Heated under pressure to 140° with dilute sulphuric acid, the catechins yield catechol and phloroglucinol. Acacatechin is disintegrated to maclurin by the action of *Penicillium solitum*. (Hazleton and Nierenstein, *J. Amer. Chem. Soc.*, 1924, **56**, 2100.)

With diazobenzene chloride the catechins give red crystalline precipitates, as observed by Nierenstein and Webster (*Collegium*, 1907, 70) in the case of the catechol tannin.

By the action of boiling water the catechins are converted into a series of anhydrides:

Catechin	Not acid, does not precipitate gelatin.
↓	
Catechin-pseudotannin	Acid; precipitates gelatin.
↓	
First Anhydride	Acid; precipitates gelatin.
↓	
Second Anhydride	Insoluble in water.

The reactions involved in these processes are still obscure and are under investigation in the writer's laboratory.

TANNIN-YIELDING MATERIALS

The geographical distribution of tannin in the vegetable world has been dealt with by J. Dekker. Although the number of plants containing tannin is relatively small, this substance appears in all the groups or subdivisions adopted by botanists. Algæ, fungi, and lichens often contain it, but the mass of raw material available in such cases is not sufficient to have any commercial value. Very

few of the mosses give a positive indication for tannin. Many of the ferns contain tannin, from mere traces to 10%, but it is in the higher group of seed plants that tannin occurs abundantly from a commercial point of view. Among the *Gymnosperms* a number of plants contain tannin, notably the pine, hemlock, spruce and fir. The *Dicotyledons* furnish the largest number of plants rich in tannin. Several of this order are widely distributed from the tropics to the limits of vegetation. The ones used in commerce are chiefly tropical. Thus the *Combretaceæ*, consisting of about 240 tropical species, yield from one of them the myrobalans of commerce. The *Rhizophoraceæ* yield the mangrove bark. The *Leguminosæ* which are chiefly useful are also tropical, such as the wattle, algarobilla, ratanhia, kino, and divi-divi. Generally speaking, the chief commercial sources of tannin are found between the parallels of 30° North and South Latitude, but an exception is found in the *Fagaceæ*, which contain the oaks and chestnuts.

The following is a summary of some of the best known tanning materials, and reference should be made to the following books for more detailed accounts: J. Dekker, *Die Gerbstoffe* (1913) and A. Harvey, *Tanning Materials* (1921).

Algarobilla (Algarobilli, Algarobillo, Algaroba) consists of the pods of *Cæsalpinia brevifolia*, Baill of Chili. The tannin-content is given as 60–68%. The tannin consists of ellagitannin and ellagic acid (A. G. Perkin, *J. Chem. Soc.*, 1897, **71**, 1131). For investigations of algarobilla see: Zölffel, *Ann.*, 1891, **229**, 123; Hartwich, *ibid.*, 1880, **216**, 281 (who gives the tannin-content as 64%); Arnaudon, *Chem. Zeit.*, 1894, 1241; Godeffroy. *Zeitg. Osterr.* Apoth-Ver., 1879, 132 (tannin-content given as 60%); Evans, *Pharm. J.*, 1887, 63.

Algarobilla gives the following reactions:

Sulphuric acid
Hydrochloric acid —render the solution turbid.
Oxalic acid

Alkali hydroxides—liquid turns brown, slight precipitate.

Lime water—darker colour, with an abundant dirty white precipitate.

Alum
Stannouus chloride —yellow-brown precipitate.

Aluminium acetate—copious clear yellow precipitate.

Lead acetate—greenish-yellow precipitate.
Copper acetate—reddish-brown precipitate.
Iron salts—blacken solution; bluish-black precipitate.
Iron salts (in small quantity)—greenish-black colour.
Potassium dichromate—yellowish-brown liquid.
Gelatin—copious yellow-brown precipitate.

Canaigre consists of the ground roots of *Rumex hymenosepalum*, Jorr, growing in the southern parts of North America. The root, which is also known as Raiz del Indio, contains 17–23% of a tannin, which differs from the tannin present in catechin-yielding plants (Richardson, *Amer. J. Pharm.*, 1889, 264).

Chestnut *Castanea vesca* Gaertn. = *Castanea vulgaris Lam.* abounds in many districts. The tannin-content is given as 7–8% (Trimble, *Chem. News*, 1892, **67**, 7). An extract is made from the wood which is chiefly used for sole leather. It is readily soluble at 60°. Sulphites and soda have been added, but they are not desirable additions. The best extracts are rich in tannins, and yet remain clear. Non-tans are said to be important in the tannin operations, but this matter is in doubt.

Particulars of the manufacture of chestnut extract in America are given by Kerr, (*J. Amer. Leather Chem. Assoc.*, 1910, **5**, 485) whereas Pawlovitch (*Collegium*, 1923, 277) gives a detailed account of the manufacture of chestnut extract in Russia. The analysis of chestnut wood is said (Alsop, *J. Amer. Leather Chem. Assoc.*, 1909, **4**, 95) to give difficulty.

Jean (*Compt. rend.*, 1902, **135**, 536) has shown that chestnut extract liberates iodine from a solution of iodic acid, and this has been used by him as a qualitative test for the detection of chestnut tree extract in oak extract.

Cutch.—(1) *Indian Cutch* and *Burma Cutches* are the dried extracts from the heart-wood of *Acacia catechu* and allied species. Prain (*J. Asiatic Soc.* of Bengal, 1897, **46**, 508) gives the distributions of these cutch-yielding plants as follows:

(*a*) **A. catechu,** *Willd.* (proper). This is the most Northern form, having been recorded as met with in Hazara, Kashmir, Simla, Kangra, Garhwal, Mussoorie, Central India, Bihar and south to Ganjam. It has never been found in the Eastern Himalayas, nor in Assam, and it has only once been reported as met with in Burma, viz., at Pegu, where according to Kurz it is called *sha*. This is therefore the

kath-yielding form of Kumaon, or *papri khair*, as it is sometimes called.

(*b*) **A. catechuoides,**—*Benth.*, is met with in Bengal from Monghyr and Patna to Sikkim, Assam and Burma. Though quite common in Pegu and Prome, it has not as yet been collected in the Shan Hills nor in Upper Burma to the north of Ava. This is therefore the cutch-yielding form of Burma; and Pegu or Rangoon cutch is the chief commercial form of the extract.

(*c*) **A. sundra,** D. C. Prain distinguishes this as the Southern and Western plant which affords the cutch of Madras and Bombay Presidencies. It is very common from Coimbatore northwards to the Deccan, Kangra and the Konkan, and has been recorded as far towards the north-west as Kathiawar and in Rajputana, also to the north-east in Burma, at Segain, Mandalay and the Shan Hills. It is the *lal khair* or Red Catechu.

The catechin and tannin content of these cutches varies greatly. Hooper (*Agricultural Ledger* 1906, 43), who has examined 26 Indian and 34 Burma cutches, gives the tannin content from 14.1 to 54.8% and the catechin content from 0 to 40.8%. Several Indian cutches examined in the writer's laboratory were found to contain from 6 to 40% catechin, but on one occasion as much as 76% catechin was found by the writer (Nierenstein, *J. Chem. Soc.*, 1922, **121,** 608).

Gambier or *Cube Gambier* is the extract of the leaves of *Uncaria gambier* and allied species. Hough (*Collegium*, 1915, 343) gives the tannin content of cube gambier as 35.0%.

Borneo Cutch is the extract made from a species of Borneo mangrove, the so called *tengah bark* or bastard mangrove (Borges, *Agric. Bull. Straits and Fed. Mal. St.*, 1904, 176). It contains 58.2% tannin.

Indian cutch is not infrequently adulterated, starch, sand, clay, and blood being among the materials said to be employed for the purpose. Jessart states he has met with an admixture of 60 to 70% of iron carbonate. Hooper (*loc. cit.*, 44) gives also a list of plants which are used as adulterants of cutch. Catechu should not yield more than 5% of *ash,* nor contain more than 12% of matter insoluble in boiling alcohol. *Starch* may be detected by treating the sample with alcohol, boiling the insoluble residue with water, and testing the cold solution with iodine, which gives the well-known blue colour in the presence of starchy matters. The presence of *ordinary tannin-*

matters is indicated by the modified colour which the sample gives with ferric salts, pure catechu giving a decided green. *Blood* may be detected by treating the sample with alcohol, and drying and heating the residue in a tube, when ammonia and strong smelling vapours will be produced. Aqueous solution of catechu should give with albumin or gelatin an abundant precipitate; with salts of tin and lead, yellow precipitates of various tints; and a brown precipitate with potassium dichromate. It should take a decided brown hue with alkalies, and give a greenish colour with ferric chloride. Good catechu yields at least half of its weight to ether and should be entirely soluble in boiling water, the latter solution depositing catechin on cooling. Catechu does not wholly dissolve in cold water unless it has been previously modified by age or exposure to dampness.

An aqueous solution of cutch gives a dense precipitate with bromine-water or cupric sulphate, neither of which reagents affects gallotannin, gallic, or pyrogallic acids. In dilute solution, Pegu cutch does not form a precipitate with potassium dichromate, but gallotannin does.

The tannin content of cutch is best estimated by Chapman's cinchonine method (page 180).

In order to distinguish between Indian cutch and gambier, Dieterich (*Pharm. Central.*, 1896, **2**, 855) recommends the following test: 3 grm. gambier are dissolved in 25 c.c. normal potassium hydroxide, and 100 c.c. water. 50 c.c. benzene (sp. gr. 0.700) are then added, and the whole is shaken in a separating funnel. After standing, the layers separate, and it is seen that the benzene shows a more or less intense green fluorescence. Acacia catechu does not show this reaction.

R. P. Biggs (Analyst, 1924, **49**, 379) recommends extracting 1–2 grm. of the extract with 7–10 c.c. chloroform in a boiling water bath. Gambier cutch gives a green solution owing to the presence of chlorophyll, whereas Indian cutch gives no coloration whatever.

Divi-divi is composed of the bean-like pods of *Caesalpina coriaria*, a small tree found in the neighbourhood of Maracaibo and other parts of South America. The pods are about 3 in. long, brown or blackish in colour, and generally folded up, or bent into the shape of a letter S. The best pods are thick and fleshy, and of a comparatively. pale colour. Deep, brown pods with black patches have been

gathered when wet, or subsequently exposed to dampness, which injures them considerably.

The dried pods contain 40–45% of a pyrogallol tannin, mainly ellagitannin, and would be a most valuable tanning material, but for a liability to fermentation and sudden development of a deep red colouring matter. The causes are not well understood, but apparently the risk can be materially lessened by the use of antiseptics.

Galls is a generic name applied to excrescences on plants produced by the punctures of insects for the purpose of depositing their eggs. Galls are the most valuable and important of all tannin matters. (See Mitchell: Section on **Inks**, p. 205.) *Nut galls, oak galls, Alleppo galls* or *Turkey-galls* are the product of the female of an insect called *cynips* (gall-wasp), which pierces the buds on the young branches of the *Quercus infectoria* and other species of oak. The eggs therein deposited soon hatch, while the bud loses its natural growth and swells out to the size of a hazel-nut. When perfect, the insect punctures a hole and escapes. Good gall-nuts should not be so pierced; they should be heavy, and of a fresh green or blue shade ("green galls"). If the insect has escaped they are yellow and inferior ("white galls"). The best oak-galls contain 50 to 60% of gallotannin, and about 3% of gallic acid.

Worm-eaten galls are sometimes doctored by filling the holes with wax. The fraud may be detected by immersing the galls in boiling water, which melts the wax and renders the holes visible. Exhausted galls have been coloured by washing them with a solution of ferrous sulphate, which is readily detected by its chemical reactions.

Knoppern are galls formed from immature acorns of several species of oak, and are used for tanning throughout Austria. In a large number of samples of Austrian galls of the year 1884, Eitner found from 28 to 35% of tannin, the moisture being about 12% (*J. Chem. Soc.*, 1885, **48**, 947).

Chinese and *Japanese gall-nuts* are a production of the *Rhus semialata.* They are very light and hollow, and distorted by numerous protuberances, and are completely covered by a thick velvety grey down. Chinese galls are much used for the preparation of tannin, of which they contain about 70%. English galls from the common oak are much inferior to the foreign varieties. They are smooth, brown, and slightly speckled with pale brown excrescences. The Japanese are smaller, paler, and generally more esteemed.

Kino generally occurs in irregular black fragments, but it is also met with in round cakes. Thin slices are often transparent and of a reddish colour; the powder is also red. Kino should be completely soluble in hot water, forming a red liquid, which, however, gradually becomes turbid. Kino is sometimes adulterated, the usual additions being dragon's blood, pitch, catechu, and ratanhia extract. The last substance may be distinguished from kino by touching a fragment of the sample with the tongue; kino remains reddish-brown, but ratanhia extract takes a fine bronze tint, so long as the surface is wet. The ash of kino should not exceed 3 or 4%.

Kino is derived from a number of plants as follows: (1) *Pterocarpus Marsupiam* Roxb (Malabar Kino). Tannin-content has been found to be 70 to 82% (Hooper, *Pharm. J.*, 1900, **1**, 226). It is said that the Kino from *Croton draco* Schlecht, resembles Malabar Kino. The extract of *Butea frondosa* is also known as Malabar Kino.

(2) *Pterocarpus erinoceus* Poir (West African Kino). Tannin-content is 7.5% (Bechholz, *Diss.* Dorpat, 1884).

(3) *Pterocarpus draco* L. (West Indian Kino). Tannin-content is 34% (Trimble, *Amer. J. Pharm.*, 1895, **67**, 78).

(4) *Pterocarpus Bussei* Harms (East African Kino).

(5) *Australian Kinos.* The following tannin-content is recorded by Blockey (*J. Soc. Chem. Ind.*, 1902, **21**, 158), for the following best known Australian Kinos:

"Iron bark" Kino (*Eucalyptus siderophlora*)	73.2% tannin.
"Ribbon gum" Kino (*E. amydalina*)............	64.8% tannin.
Kino from *E. piperita*........................	31.5% tannin.
"Bloodwood" Kino (*E. corymbosa*)..............	30.3% tannin.
"Grey gum" Kino (*E. punctata*)................	38.3% tannin.
Kino from *E. stillata*........................	31.6% tannin.

Mallet Bark (*Eucalyptus accidentalis*, Endl) also known as *Mallets Bark*. This bark, which has been on the market since 1905, came from Australia.

The tannin content is 35–52%. The non-tannin-content is 7–16% (Paessler, *Collegium*, 1906, 58 and 65). Only the flesh of the bark is used, the *ross* being of little value. The tannin is easily soluble, 95% of it dissolving in cold water. The solutions keep well and give a satisfactory colour to leather.

The tannin has been investigated by Dekker (*Arch. Neérland Sc. Exact. Nat.*, 1909, (2) **14**, 50).

Mangrove.—Red Mangrove (*Rhizophora mangla*, L.) grows in the mud by the sea; the wood is very hard, the heart being dark red. The young wood is yellow. The bark is said to contain 17.5% tannin (Moller) or 39% (Paessler), and is used locally for colouring fishing nets. This red colour is an objection in tanning.

Trimble (*Contrib. Labor. Bot. Univ. Penn.* 1892, **1**, 50) obtained the following reactions with a 1% solution:

Ferric chloride	Dirty green ppt.
Lime water	Pink ppt.
Bromine water	Yellow ppt.
Uranium acetate	Red-brown colour.

No sugar was found associated with the tannin, which is a catechol derivative. It is said to be the only tannin which gives a precipitate with sulphuric acid.

White Mangrove (*Laguncularia racemosa*, Gr.) contains a pyrogallol tannin. It is said to be useful in combination with divi-divi, producing a light leather.

There will probably be an extended use for this in the future. The composition of these 2 varieties has been given as follows (Nierenstein and Webster, *Quart. J. of Inst. of Comm. Research in the Tropics*, 1908, **3**, 6):

	White mangrove, %	Red mangrove, West African, %
Water	10 42	10.42
Tannins	22.80	9 10
Org. non-tannins	3.06	13 64
Inorganic matter	2.95	2 52
Insol. at 100°	62.77	64 32

White Mangrove contains a yellow colouring matter, "Languncurin" (Nierenstein and Webster, *loc. cit.*).

Busse (*Arbeit. K. Gesundheitsamt.*, 1899, **15**, 177) has estimated the tannin-content of a number of mangroves, with the following results: *Rhizophora mangla* L., 42%; *R. Mucronata*, Lam., 48%; *R. opiculata*, Blme. (=*Bruguiera gymnorrhiza*, Lam.), 51.6%; *Bruguiera parviflora*, Wght. and Arn., 42%; *Sonneratia caseolaris*, L., 15.5%; *Heritiera littoralis*, Dryand., 13.9%; *Xylocarpus granatum*, Koen, 40%.

The solid extract contains 12% water, 68.5% tannin and 17.3% non-tannins. Unless the extract is to be used as a dye it is decolorised by blood albumin. The Queensland variety contains 39% tannin; the Madagascar variety, 43–44%; Celebes, 45–48%; E. Africa, 38–42%. As the outcome of a great number of analyses (Paessler, *Der. Pflanzer*, 1912, 65) of German East African samples the following results were obtained for the tannin present:

	Lowest, %	Highest, %	Mean, %
Rhizophora	29.3	40.8	36.5
Bruguiera	24.8	42.3	35.8
Ceriops	24.2	32.2	25.8
Xylocarpus	26.7	32.5	29.8

The average quantity of water present was 14%. The absence of sodium chloride in an extract precludes the presence of mangrove (Lauffmann, *Collegium*, 1913, 119), although its presence may be due to other causes.

Avincennia nitida, Tacq, known as "couridabark" in British Guiana, has wrongly been described as *mangrove* (Peckolt, *Ber., Deutsch. Pharm. Ges.*, 1904, 372), as it contains very little tannin, and it is possible that *Avincennia tomentosa*, L., which is known as "mangle prieto" in the East Indies, accounts for this statement. The latter contains 18% tannin.

Mimosa or Wattle Bark.—This tanning material which is obtained from various species of acacia, contains a catechol tannin. First grown in Australia, it has, since about 1880, been introduced into Africa and Ceylon, where its cultivation has become of extreme importance. The tannin content for different Australian species is given as 12.2 to 49.5%, and similar data have been recorded for the same species introduced into Africa.

Myrobalans are the fruit of several species of *Terminalia*. In size and shape the myrobalan resembles a slightly shrivelled plum. As imported, myrobalans contain from 3 to 7% of moisture, and leave about 10% of ash on ignition. The tannin is chiefly contained in the dried pulp enclosing the stone. The tannin-content is 27.3 to 33%.

Good myrobalans should be of a pale buff colour, plump, or but slightly shrivelled, and free from worm-holes or blackish stains or blotches. They should be hard and firm, and when broken with a

hammer should form a light-coloured dry powder and irregular fragments. If they crumble between the fingers to a dark coloured dust, or flatten under the hammer, they are inferior. The stones contain very little tannin, and hence their proportion should be ascertained by breaking 50 nuts with a hammer, clearing the stones from any adherent pulp, and weighing them separately. They may constitute from 23 to 52% of the whole fruit.

Ground myrobalans should be light in colour, dry, and free from a saline, or an intensely bitter taste. When slightly moistened and rubbed in the hand they should adhere tenaciously to the skin.

Myrobalans are sometimes mixed with earth, sand, *nux vomica*, betel-nuts, and a variety of seeds and berries. They may also be adulterated with finely ground divi-divi, wild galls, and old and worthless sumach. On scattering the powdered substance on a sheet of paper, and examining it with a lens, it will be recognised by portions of its brown, flat, smooth pea-like seeds, which from their hardness and smoothness escape being crushed to powder. The leaf stalks of sumach are readily distinguished from the torn, irregular fibre of the myrobalans.

Oak bark can be said to be one of the oldest materials used for the manufacture of leather, but owing to its low tannin content (10–14% tannin), and the introduction of other materials and extracts, it is now coming into disuse to a large extent.

Oak-wood contains only about 6%, but is used in the manufacture of oak-wood extract. The extract contains 25–27% tannin. According to Jedlicka (*Collegium*, 1913, 33) oak extract contains 0.6–1.3% acetic acid.

Owing to the high sugar content of its extract it is liable to fermentation (Thuan. *Le Cuir*, 1913, 595).

Oak extract is frequently adulterated with chestnut extract. Jean (*Compt. rend.*, 1902, **135**, 536) recommends the following method for the detection of chestnut extract in oak-extract: The oak extract is repeatedly shaken with aqueous solution of iodic acid and carbon disulphide, chloroform, benzene or carbon tetrachloride, the latter being separated in the usual way. The liberation of iodine shows the presence of chestnut wood extract, and the quantity of iodine can be estimated by titration; 1 part of iodine corresponds with 6.25 parts of dry chestnut wood extract, 19 parts of extract of 20° Bé., or 16 parts of extract of 25° Bé.

Palmetto (*Seremoa serrulata,* Hook) is an evergreen palm-like shrub, which grows abundantly in the southern states of America.

The aqueous extract, commercially known as "Palmetto-Extract," contains from 5 to 12% tannin and is used in industry.

Quebracho (*Aspidospera quebracho,* Claneo Schlecht) is a tree which is native of Argentina, Cuba, and other sub-tropical countries. Its name signifies "break hatchet," and refers to the great hardness of the wood. The tree grows to a considerable size, and its bark is thick and red and possesses important tanning properties. The wood has a density of 1.26, and when freshly cut has a bright orange colour which rapidly darkens to a reddish shade on exposure. The tannin is readily extracted from the bark and wood by boiling water. The ground wood contains about 18% tannin, which, however, is reduced on exposure to the air for any length of time. The extract, 30° Bé., contains about 50–56% of tannin, while the dried extract will contain over 60% of tannin.

An infusion of quebracho gives the following reactions:

Dilute sulphuric acid and hydrochloric acid, a bright orange precipitate.

Alum to an alkaline solution, an orange-red lake.

Aluminium acetate, a yellow precipitate.

Stannous chloride, an orange-yellow precipitate.

Stannic chloride, a darker precipitate.

An iron salt, in small quantity, a blue-black precipitate.

An iron salt, in large quantity, a grey precipitate.

Potassium dichromate, a reddish-brown precipitate.

Copper salts, a greenish precipitate.

Lead acetate, a bright precipitate.

Aluminium sulphate, a greyish precipitate.

Quebracho Extract.—A normal liquid extract of this wood contained 34.5% tannin, 3.5% non-tans.: water 60%, dextrose 0.3%, sucrose 0.2% and ash 1.0%. An extract showing high non-tans. (6.5%) also gave a high ash (3.0%). In a special case an extract giving 28.7% tans., 10.7% non-tans., 12.2% dextrose, and 2.9% ash was regarded with suspicion, and a qualitative test indicated the presence of myrobalans, (Paessler). Schell (*J. Amer. Leather Chem. Assoc.* 1912, **7**, 564) has suggested a special test to detect mangrove in quebracho extract.

The ratio of insoluble to soluble tans. is given by W. Möller (*Ledertechn. Rundschau*, 1913, **5**, 258) as 1:10 at analysis strength. It increases on further concentration to 2:3 at 8° Bé., but on continuing the concentration to 20° Bé., solution is complete.

The adulteration of quebracho extract by mangrove may, according to van Gijn and van der Waerden, (*J. Amer. Leather Chem. Assoc.*, 1914, **9**, 109) be detected by estimating the pentoses and pentosans present in the extract by the usual Tollens-Krober method of estimating furfural. Quebracho extract is almost free from pentosans and pentoses, while mangrove contains fair quantities of these substances. Details of the method of estimation may be found in the original communication. W. Möller (*Collegium*, 1914, 85) criticises this method.

Lauffmann, on the other hand, proposes to precipitate the tannin by his ammonium molybdate method. With untreated quebracho extract the Mo-figure varies from 28 to 37, but unfortunately in sulphited quebracho it varies from 5 to 37. Mangrove extract gives a figure between 120–130 and sulphited mangrove extract 111. Stiasny (*Collegium*, 1914, 77) has confirmed the fact that mixtures of these two extracts act satisfactorily in this test, but it must be noted that if a Mo-figure of 30 be taken as a standard for quebracho extract, and anything above this be regarded as due to added mangrove extract, an error equal to 20% of mangrove may be looked for if the quebracho extract is a sulphited extract giving an actual figure of 5. In making this test care must be taken to see that no pyrogallol-tans (formaldehyde test) or sulphite cellulose (aniline test) be present. Stiasny and Wilkinson have shown that an additional sulphite process in the laboratory to equalise the original quebracho extracts to a common sulphite basis is unsatisfactory. They consider that further work will be necessary before this test can be considered as authoritative. G. E. Kerr (*J. Amer. Leather Chem. Assoc.*, 1914, **9**, 27) proposes to identify the addition of mangrove by a phloroglucinol test: 100 c.c. of hydrochloric acid (12%) are placed in a 250 c.c. Erlenmeyer flask, and 2 grm. of the tannin material added in the dry state (or an equivalent amount of extract) with a few pieces of pumice stone to prevent bumping. The solution is distilled through an ordinary glass condenser at the rate of 30 c.c. every 10 minutes, 30 c.c. of hydrochloric acid being added through a thistle head tube as each 30 c.c. distils over. Distillation is carried

on until 300 c.c. have passed over. The distillate is tested by placing 100 c.c. in a glass beaker (2 in. diam.) and adding 8 c.c. of a solution of phloroglucinol (made by dissolving 0.25 grm. in 25 c.c. of 12% hydrochloric acid). The solution is stirred for a few moments until the colour reaches its maximum (within 5 minutes). This remains permanent for some time. With pure quebracho the colour is first a brilliant yellow, gradually becoming a bright green and finally a bluish tint as the precipitate forms, which is a dense black. With mangrove the first colour is orange, developing to a deep orange, and the precipitate is buff-coloured instead of black. In mixtures of quebracho and mangrove it is claimed that even so low an addition of the latter as 5% will change the colour to olive. At 50% the green colour is dominated by the orange of the mangrove. Under a low power, the precipitates may be seen side by side when small additions of mangrove are present.

Sumach or **Sumac,** consists of the leaves, leaf-stalks, and small twigs of several species of *Rhus*. It is sometimes sold whole, sometimes coarsely bruised, but more commonly in fine powder. The best Sicilian sumach gives a bright green powder which has a pleasant tea-like odour. The second quality is reddish-yellow; and Spanish sumach has usually a fawn colour.

Sumach should be quite dry, and free from cakes or lumps, the presence of which shows that the sample has been exposed to dampness and will probably have become seriously deteriorated. The colour should be bright. If dull, the sample is probably damaged by long keeping, or is mixed with sumach of inferior quality.

Sumach sometimes contains a notable proportion of earth or sand. 10% of ash is sometimes left on ignition.

Cape sumach (*Osyris compressa*) contains a glucoside osyritrin. Transvaal sumach (*Osyris abyssinica*) is said to produce an inferior leather and colour. This tannin forms a phlobaphene more readily than the Cape variety. It also contains osyritrin.

The analysis of Virginia sumach has been considered in some detail by Palassay (*J. Amer. Leather Chem. Assoc.*, 1910, **5**, 404).

In the detection of sumach in the state of leaf the microscopical examination of the leaf cuticle is of great value. This was originally pointed out by Lamb (*J. Soc. D. and Col.*, 1904, **20**, 265). When the sumach leaves are adulterated they are generally supplied in a half or wholly ground condition, and therefore a microscopical

examination is necessary to disclose the structure; a 1 in. objective is sufficient to detect the difference between sumach and adulterant material after the treatment recommended by Lamb. The most common adulterant is the leaves of *Pistacia lentiscus*, which grow abundantly in Cyprus. It has even been stated that some 10,000 tons of this material are used annually to adulterate sumach. The *Tamarix africana* is also used for this purpose. Lamb found that not more than 10% of a number of samples were unadulterated. His method of procedure is as follows: 1–2 grm. of the sample are placed in a large boiling tube and covered with nitric acid (1:1). The mixture is well shaken to wet the sumach thoroughly, and the tube gently heated over a small Bunsen flame until nitrous fumes are evolved. The tube is then left to stand for 15–30 minutes. At the end of this period the tube is again heated until the solution becomes quite clear. An excess of water is then added and the mixture filtered through a filter paper of close texture, and the residue washed with distilled water. A small hole is made in the filter paper, and the residue washed through into a basin with distilled water. A few drops of a solution of dyestuff are added and the mixture gently warmed for a few minutes until the small particles are coloured but not so deeply as to lose their transparency. Bismarck brown, safranine, and methylene blue are suitable for the purpose. After removal of the surplus dye solution the particles are filtered off, as before, through paper, washed with a little water, and a hole once more made in the filter paper and the residue washed into a clean porcelain basin. A number of the dyed particles are transferred to a microscope slide and a cover-glass placed over them. A reference to the original paper will show the considerable difference observed between the true sumach and the adulterants. The cellular structure of the adulterants is quite distinctive; the stomata afford a valuable means of identification. The treatment with nitric acid, if prolonged, dissolves the cuticle of sumach and leaves nothing more than what has been described as a "wreck," whilst the adulterants are not acted on in this manner.

Valonia.—(*Valonen, Vallonen*) consists of the acorn cups of certain species of oak (*Quercus ægilops*, *L. Q. Vallonea* Kotsch and *Q. græca* Kotsch). They contain gallotannin, gallic acid, ellagitannin and ellagic acid (Stenhouse, *Annalen*, 1843, **45**, 7; *Zeitsch. anal. Chem.*, 1875, **14**, 46; Jahn, *Ber.*, 1875, **8**, 2107; Eitner, *Der Gerber*,

1876, 430; Bötinger, *Arch. Pharm.*, 1895, **233**, 125). Valonia is chiefly exported from Smyrna, but also obtained from other parts of Asia Minor, Greece, the Grecian Archipelago and Canada. Procter (*The Principles of Leather Manufacture*, 258) gives, for best Smyrna valonia, 40% tannin; Greek valonia, 19–30% and Canadian valonia up to 41% tannin. Valonia should be of a bright drab colour. If dark, it has suffered from dampness and will be inferior in quality.

White tan (*Cæsalpinia digyna*, Rottl), also known as Tari or Teri, contains 30–50% tannin. The tannin-bearing pods grow well in Burmah.

An analysis of the pods by Pilgrim ("*Indian Tanstuffs*," Calcutta, 1920) gave 41.51% tannin, 24.83% soluble non-tannins and 33.66% insoluble matter. Pilgrim found 10% moisture in the air-dry raw material.

The following data are given by G. Savani and T. Torquati. (*Boll. Inform. Comm. del Min. delle Colonia*, 1923, 28) for tanning materials from the Italian Somaliland:

Botanical name	Common name, etc.	Tannin, %	Soluble non-tans., %	Insoluble Matter	moisture, %	Ash in soluble matter, %
Fiscus Sicomorus...........	Mocoi bark	9.91	8.09	.....	9.15	14.40
Poinciana Regia............	....	7.88	4.97	.....	8.30	5.60
Acacia Adansonii..........	Bark	14.10	8.51	.....	9.33	5.30
Acacia Adansonii/........	Bark	10.90	3.90	.....	9.30	5.90
Acacia Adansonii..........	Pods	23.20	19.87	.....	8.85	6.90
Acacia Adansonii..........	Immature pods	16.86	23.97	.....	9.61	5.50
Acacia Adansonii..........	Root bark	21.12	10.40	.....	9.60	5.30
Acacia Adansonii..........	Trunk bark	12.03	9.54	.....	9.75	7.01
Acacia Seyal..............	Fullaj	7–8	10–11	.....	.....	......
Acacia Stenocarpa.........	Dammai bark	8.31	5.77	.....	.....	......
Acacia Bussei, Harms.....	Bark	9.32	3.32	.....	10.25	4.70
Acacia Bussei, Harms......	Bark	17.46	6.70	.....	9.80	4.30
Acacia Bussei, Harms......	Bark and root	18.82	8.83	.....	9.10	4.05
Acacia Bussei, Harms......	Bark	23.97	11.60	.....	8.79	2.70
Acacia Bussei, Harms......	Bark	11.92	10.16	.....	7.90	7.30
Acacia Bussei, Harms......	Roots	6.61	5.45	.....	9.01	5.50
Acacia Bussei, Harms......	Bark	14.51	10.05	.....	8.10	3.00
Acacia Bussei, Harms....	Bark	19.97	6.60	.....	8.15	4.40
Acacia Bussei, Harms..	Roots	7.86	3.80	.....	8.05	7.90
Acacia Stephanini.....	Bark	5 52	4.98	.....	8.80	10.80
Acacia Stephanini... ..	Bark	9 44	3.89	.....	.....	......
Acacia Stephanini.... ...	Bark	16.82	5.47	.....	8.25	3.90
Acacia Stephanini	Bark	16 28	7.60	.....	8.50	3.30
Acacia Stephanini..........	Roots	9.67	4.64	.....	8.72	5.90
Acacia Stephanini........	Root-bark	10 97	2 85	....	....	.....
Carsia abbreviata (Lin.)....	Bark	13 24	13 26	...	8.40	6.50
Ceira pentandra (Gart.) ...	Bark	4.50	. .	.....	....	...
Terminalia (Respoli) (Englei)...	Bark	4.80	7 59	.	. ..	. .
Mimusops densiflora (Englei).........	Bark	8.13	7 19		....	.. .
Kigelia Ætiopica..........	Bark	5 27	11.17		. .	... .
Cassine Æquifolium (?)	Bark	10 00	9 32			..
Cassine Aequifolium (?)	Kausan Bark	24.32	8.72	.	12 72	3 40
Cassine Aequifolium (?)	Agar Bark	15.8	4.90		8 90	6 10

QUALITATIVE RECOGNITION OF TANNIN MATERIALS

General Tannin Tests.—(1) *The Gelatin Test:* Tannins are precipitated by gelatin. The gelatin-solution is prepared by dissolving 1 gr. of gelatin in 120 c.c. of water *which is slowly warmed in a water-bath at* 70°. It is advisable to add a little chloroform to the solution so as to prevent infection. Only dilute tannin solutions should be used. Alcoholic tannin solutions should not be used, as alcohol produces a turbidity when added to the aqueous solution of gelatin. Although generally accepted as a specific tannin test, it must be remembered that gelatin is also precipitated by (1) gum arabic

(Pelletier, *Ann. Chim.*, 1813, **87**, 106), (2) starch and inulin (Tollens, *Kurzes Lehrbuch der Kohlenhydrate*, 525, 551), and (3) methyl gallate (Nierenstein, *Collegium*, 1905, 307; *Ber.*, 1912, **45**, 837). In this connection it must be noted that methyl-gallate does not give the goldbeater's skin test for tannins (Price, *Analyst*, 1924, **46**, 25).

(2) *Alkaloids* precipitate tannins. Here, again, it must be remembered that other phenolic substances are also precipitated by alkaloids, for example: phenol (Cotton, *Bull. Soc. Chim.*, 1875, (2), **24**, 535) and salicylic acid, both of which form precipitates with quinine (Jobst, *Jahresber.*, 1875, 769).

(3) Tannins give deep coloured solutions or precipitates with *iron salts*, and this property is shared by other phenolic substances.

(4) Tannins give coloured precipitates with *potassium chromate.* Drabble and Nierenstein (*Biochem. J.*, 1907, **2**, 96) found, however, that gallic acid is also precipitated by potassium chromate.

The above-mentioned tests are generally considered to be specific tests for tannins, but this is, however, obviously not the case.

As far as the writer is aware, the only specific tannin test known is the goldbeater's skin test, which is described on page 78.

Special Tannin Tests.—The following tables, due to Procter, show the behaviour of infusions of a number of commercial tannin matters with various reagents. The infusions must be very weak, not exceeding 1.002 sp. gr., or precipitates may be formed where mere coloration or clouding is described as occurring. By means of the table, the origin of any simple tannin infusion is said to be ascertained, but in the case of mixed infusions the indications are less reliable. In such cases, colour reactions are misleading, and it is safer to rely on the direct test of precipitate or no precipitate, coloration or no coloration, without regard to the tint.

In some cases, only negative indications are recorded, and the material cannot be positively identified in admixture with other tannin matters giving positive indications with the same reagents. Thus an infusion of myrobalans could not be distinguished with certainty from an infusion of divi-divi, where any other material, such as gambier, was present, which gives a deep coloration with concentrated sulphuric acid.

In addition to the reactions described in the table, the identification of the products of the action of heat on tannins, and of their treatment with dilute acids and fusing alkali hydroxide, affords a valuable means of identification.

Most, if not all, of the ordinary varieties of tannin give with an ammoniacal solution of potassium ferricyanide a deep red coloration, rapidly becoming brownish, especially on addition of excess of the reagent.

The extract from a bark yields more ash than that from a wood.

Reagent	Myrobalans	Divi-divi	Valonia	Oak-bark	Chestnut wood (extract)
Boiled with equal volume of dilute sulphuric acid (1 to 9).	Pale deposit (ellagic acid) on cooling.	Pale deposit (ellagic acid) on cooling.	Slight pale deposit.	Slight pale deposit or turbidity on cooling.	No deposit.
Bromine water.	No precipitate.	No precipitate.	No precipitate.	Pale precipitate.	No precipitate.
Dilute ferric chloride.	Blue-black precipitate.	Dark blue precipitate.	Blue-black precipitate.	Bluish-black precipitate.	Blue-black precipitate.
On adding ammonia.	Brown precipitate.	Dark red precipitate.	Red-brown precipitate.	Red-brown precipitate.	Purple precipitate.
Solution tartar emetic.	No precipitate.	Faint clouding.	No precipitate.	No precipitate.	Slight clouding.
Add ammonium chloride.	Light precipitate.	Dense precipitate.	Pale precipitate.	Whitish precipitate.	Pale precipitate.
Copper sulphate.	Faint clouding.	Slight green precipitate.	No precipitate.	Slight precipitate.	No precipitate.
On adding ammonia.	Dense dark precipitate.	Dense dark precipitate.	Dark reddish precipitate.	Brown precipitate.	Light brown precipitate.
Lime-water.....	Yellow precipitate turning greenish.	Yellow precipitate turning purple.	Yellow precipitate turning red purple.	Brown precipitate.	White precipitate turning light blue.
Ammonium molybdate in nitric acid.	Dirty yellow precipitate.	Dark greenish precipitate.	Dark greenish precipitate.	Greenish precipitate.	Yellow colour.
With sodium sulphide exposed to air.	Yellow colour.	Yellow colour.	Turns purplish-red.	Turns red.	No change.
Add concentrated sulphuric acid to 1 drop of infusion.	Yellow colour.	Intense crimson.	Deep yellow.	Deep red precipitate on dilution.	Light yellow.
Lead nitrate.	Light yellow precipitate.	Dark yellow precipitate.	Pale precipitate.	Brown precipitate.	White precipitate.
Cobalt acetate..	Buff precipitate.	Buff pink precipitate.	Dirty pink precipitate.	Brown precipitate.	Flesh-coloured precipitate.
Manganese acetate.	Yellow precipitate.	Yellow precipitate.	Dirty yellow precipitate.	Brown precipitate.	White precipitate.
Uranium acetate.	Dark red colour.	Dark red colour.	Dark red colour.	Dark brown precipitate.	Crimson colour turning dark red.
Ammoniacal picric acid solution.	No precipitate.	No precipitate.	Brown precipitate.	No precipitate.	No precipitate.
Potassium dichromate.	Brown precipitate.	Brown precipitate.	Brown precipitate.	Brown precipitate.	Brown precipitate.

Hungarian larch (extract)	Hemlock (extract)	Mimosa bark	Cutch (Pegu)	Gambier (cube)	Gallotannic acid, 1 %
Yellow flocculent deposit separates quickly.	Abundant red flocculent deposit.	Heavy red deposit on cooling.	Light red deposit on cooling.	Reddish deposit on cooling.	Usually some pale deposit.
Yellow precipitate.	Yellow precipitate.	Yellow precipitate.	Yellow precipitate.	Yellow precipitate.	No precipitate.
Dull brown precipitate.	Dirty green precipitate.	Full brown precipitate.	Green-black precipitate.	Intense green colour.	Blue-black precipitate.
Dull red precipitate.	Reddened precipitate.	Purple colour.	Dark red precipitate.	Reddened.	Reddened precipitate.
No precipitate...	No precipitate.	White precipitate.	No precipitate.	No precipitate.	No precipitate.
Pale precipitate.	Slight pale precipitate.	Dense white precipitate.	Pale precipitate.	Faint clouding.	White precipitate.
Slight cloud.	Pale precipitate.	Slight precipitate.	Dense precipitate.	Profuse precipitate.	No precipitate.
Deep blue coloration.	Dark green coloration.	Deep red precipitate.	Deep violet coloration.	Dark green coloration.	Brown precipitate.
Dirty brown precipitate.	Brown precipitate.	Slight reddish precipitate.	Slight cloud, soluble in excess.	No precipitate.	Pale precipitate turning blue.
Slight clouding.	Slight precipitate.	Brown precipitate.	Slight cloud, soluble in excess.	No precipitate.	Yellow colour.
No change......	No change.	Turns red.	Slight reddening.	No change.	No change.
Dark brown or crimson.	Intense crimson.	Intense purple-red.	Deep red, no precipitate on dilution.	Dark brown or crimson.	Yellow.
Pale precipitate.	Pale precipitate.	Clouding.	No precipitate.	Faint clouding.	White precipitate.
Purplish precipitate.	Purple precipitate.	Brown precipitate.	Brown precipitate.	No precipitate.	Purple precipitate.
Slight clouding.	Slight precipitate.	No precipitate.	No precipitate.	No precipitate.	White precipitate.
Slight darkening.	Light brown precipitate.	Dark red colour.	Dark red colour.	Dark red colour.	Crimson colour. Brown precipitate.
No precipitate.	Clouding.	No precipitate.	No precipitate.	No precipitate.	No precipitate.
No precipitate.	Brown precipitate slowly formed.	Brown precipitate.	Brown colour.	Brown precipitate slowly formed.	Brown precipitate.

The ash of an oak or pine extract may contain manganese and have a green colour, or become green on being fused with sodium carbonate and a little potassium nitrate.

On shaking a concentrated solution of quebracho extract with ethyl acetate, the ethereal layer becomes at first green and then brown.

The bark and extract of the American chestnut oak (*Quercus castanea*) contains a substance exhibiting, like æsculin, a powerful blue fluorescence, especially in alkaline solution.

Sumach extracts are distinguished by a peculiar tarry smell, and yield a high percentage of ash.

Procter (*J. Soc. Chem. Ind.*, 1894, **13**, 487) gives the following tables for the qualitative recognition of tanning materials:

TABLE I

<table>
<tr><td colspan="2">Bromine water produces a precipitate
Iron-alum gives greenish-blacks
(Catechol tannins)
$CuSO_4$ with excess of NH_4OH</td><td colspan="2">Bromine water produces a precipitate
Iron-alum gives blue or purplish-blacks
(Mixed and doubtful)
$NaNO_2$ with 5 drops of N/10HCl</td><td colspan="2">Bromine water produces no precipitate
Iron-alum gives blue-blacks
(Pyrogallol tannins)
$NaNO_2$ with 5 drops of N/10HCl</td></tr>
<tr><td>Precipitate redissolves</td><td>Precipitate does not redissolve</td><td>No reaction or, at most, darkening</td><td>Colour change from red towards blue or green</td><td>Colour change through red to blue</td><td>No reaction</td></tr>
<tr><td>Class 1*a*
Table II</td><td>Class 1*b*
Table III</td><td>Class 2*a*
Table IV</td><td>Class 2*b*
Table V</td><td>Class 3*a*
Table VI</td><td>Class 3*b*
Table VII</td></tr>
</table>

TABLE II

Class 1a	Ferric alum	Bromine water	Nitrous acid	$CuSO_4$ + NH_4OH	$SnCl_2$ + HCl	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
Cutches from Ac. catechu wood.	Green-black.	Pp.	No react. darkens.	Pp. redissolves red-violet colour.	No react.	Deep violet-red.	Reddens.	Red-brown colour.	Reddish pp. slowly formed.
"Thann leaf" extract (a cutch substitute).	Olive-black pp.	Pp.	Do.	Pp. redissolves brown colour.	No react.	No react.	No react.	Crimson dilutes pink.	No pp.
"Turwar" bark (*Cassia auriculata*).	Green-black.	Pp.	Do.	Pp. redissolves red violet.	No react.	Trace.	Pink colour.	Crimson.	Reddish pp.
"Gambene" extract (a gambier substitute).	Green-black colour.	Pp.	Do.	Do.	No react.	No react.	Slight pink colour.	Crimson, dilutes pink.	Do.
"Teugah" bark (*Ceriops Candolleanna*).	Do.	Pp.	No react. darkens pp.	Do.	Pink colour.	No react.	Pink colour.	Crimson.	Bright red pp.
Bark (*Acacia leucophlœa*)	Do.	Pp.	No react.	Do.	Do.	Slow violet react.	Pink colour.	Crimson, dilutes pink.	Dull brown pp.
Bark (*Soymida febrifuga*)	Do.	Pp.	No react.	Pp. redissolves red-brown.	Do.	No react.	Pink colour.	Crimson.	Red brown.
Cork bark (*Quercus suber*).	Green-black coloration.	Pp.	Reacts somewhat.	Pp. redissolves brown.	No react.	No react.	Reddens.	Crimson, dilutes pink.	Reddish brown pp.
Green oak (Ital.) (*Quercus Ilex*).	Do.	Pp.	Reacts faintly, if at all.	Do.	No react.	No react.	Reddens.	Do.	Do.
Garouille (root bark of Kermes oak) (*Quercus Coccifera*).	Do.	Pp.	Reacts?	Do.	No react.	No react.	Reddens	Do.	Do.

TABLE II.—(*Continued*)

Class 1a	Ferric alum	Bromine water	Nitrous acid	$CuSO_4$ + NH_4OH	$SnCl_2$ + HCl	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
[1] Quercitron bark (*Quercus tinctoria*).	Green-black coloration.	Pp.	Reacts somewhat.	Pp. redissolves brown.	Light green.	No react.	Doubtful.	Crimson, dilutes pink.	Reddish brown pp.
Gambier (ext. of leaves of *Nauclea gambir*).	Deep green coloration.	Pp.	No react. darkens.	Pp. redissolves olive-green.	Yellow.	Deep violet-red.	Yellow.	Crimson, dilutes brown.	No pp.
[2] "Pruim bast" (leaves of *Colpoon* or *Osyris compressa*).	Green-black.	Pp.	No react.	Pp. redissolves green.	No react.	Pink.	Yellow.	Do.	Light yellow pp.
[3] "Koko." Natal (leaves of *Celastrus buxifolia*).	Do.	Pp.	No react.	Do.	No react.	No react.	Yellow.	Dark brown.	Bright yellow pp.
Larch bark (*Larix europæa*).	Green-black coloration.	Pp.	No react. darkens.	Pp. redissolves olive-green.	Pink coloration.	No react.	No react. darkens.	Deep red-brown.	Rusty pp.
Hemlock bark (*Tsuga* or *Abies canadensis*).	Olive-green reddish pp.	Pp.	No react., pink with $NaNO_2$.	Pp. redissolves neutral tint.	Do.	No react.	Reddens.	Crimson, dilutes pinkish.	Red-brown pp.
"Larch" extract from *Abies excelsa*.[4]	Green-black or brown.	Pp.	No react.	Pp. redissolves olive-green.	Do.	No react.	Darkens.	Deep red-brown.	Brown pp.

[1] Dyes yellow with Al and Sn mordants.
[2] Used at Cape of Good Hope as sumach.
[3] Used in Natal as sumach substitute.
[4] *Fichte, Rothtanne*, Norway or common spruce. *Abies pectinata* the *Weiss* or *Edel-Tanne* or silver fir, is said to give a blue-black colour with iron.

TABLE III

Class 1β	Ferric alum	Bromine water	Nitrous acid	$CuSO_4$ + NH_4OH	$SnCl_2$ + HCl	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
Willow bark (Russian. Sp. unknown).	Green-black.	Pp.	No react.	Dense pp.	No react.	Violet faint.	Pink coloration.	Red-brown not intense.	Slight greyish pp.
Acacia Angica or Piptadenia macrocarpa.	Do.	Pp.	No react.	Dense chocolate pp.	Pink or violet colour.	Do.	Reddens somewhat.	Crimson dilutes pink.	Reddish pp.
Acacia catechu bark.	Do.	Pp.	No react.	Dense violet-black pp.	Possible trace.	Trace.	Pink colour.	Red-brown.	Flesh colour pp.
"Thorn tree" bark (*Acacia horrida*) (Cape).	Do.	Pp.	No react. darkens.	Dense pp.	No react.	Doubtful.	Pink colour.	Dull crimson not intense.	No pp.
Mangrove bark extract (*Rhizophora mangle*).	Do.	Pp.	No react.	Reddish-black.	Slight reddening.	No react.	Slight reddening.	Red-brown.	Red pp. darkened by excess.
Quebracho wood extract (*Quebracho or Loxopterygium Lorentzii*).	Green-black coloration.	Pp.	No react.	Dense pp.	Pink colour pp.	Trace.	Doubtful.	Crimson coloration dilutes pink.	Light brown pp.
"Sugar bush" bark (Cape) (*Protea mellifera*).	Green-black.	Pp.	No react. darkens.	Dense pp.	No react.	Trace.	Doubtful.	Red.	Yellow-brown pp.
"Waagenboom" (Cape) (*Protea grandiflora*).	Do.	Pp.	Do.	Dense pp.	No react.	Trace.	Pink colour.	Crimson dilutes pink.	Light yellow pp.
"Kruppelboom" (Cape) (*Leucospermum conocarpum*).	Do.	Pp.	Do.	Dense pp.	No react.	Violet distinct.	Pink colour.	Do.	Slight greyish pp.
"Silver tree" (Cape) (*Leucodendron argentea*).	Do.	Pp.	Do.	Dense pp.	No react.	No react.	Pink coloration.	Do.	Flesh colour pp.
[1] Chestnut oak (*Quercus castanea*).	Olive-green coloration.	Pp.	Reacts. distinctly.	Decided pp. Insoluble in excess.	No react.	No react.	Reddens.	Crimson dilutes pinkish.	Reddish-brown pp.

[1] Infusions fluoresce, especially with ammonia.

TABLE IV

Class 2α	Ferric alum	Bromine water	Nitrous acid	$CuSO_4$ + NH_4OH	$SnCl_2$ + HCl	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
"Skens," cypress sumach (possibly *Coriaria myrtifolia*).	Blue-black pp.	Pp.	No react.	Dark pp.	No react.	No react.	Yellow.	Yellow-brown.	Yellow pp. darkening.
Kliphaut bark[1] (*Rhus Thunbergii*).	Blue-black.	Pp.	No react	Dense dark pp.	No react.	No react.	Pink.	Dull crimson dilutes orange.	Pinkish pp.
Canaigre (root of *Rumex hymenosepalus*).	Blue-black pp.	Pp.	No react.	Dense dark pp.	No react. clouds.	Trace violet.	Slight darkening.	Yellow-brown.	Pink coloration greyish pp.
"Talwaan" or "Elands-bontjes" (root *Elephantorrhiza Burchellii*).	Blue-black pp.	Pp.	No react. Darkens.	Dense dark pp.	No react.	Trace violet.	Pink.	Red.	Reddish-brown pp.
Mimosa or Wattle barks (Various Austral. *acaciæ*).	Dirty violet pp.	Pp.	No react.	Dense purple brown pp.	Slight reddening.	Sometimes trace.	Reddens.	Crimson dilutes pink.	Reddish or yellow-brown pp.
Babool bark. India (*Acacia Arabica*).	Do.	Pp.	No react.	Dense dark pp.	Some trace.	Faint trace.	Slight darkening.	Crimson dilutes orange.	Dark reddish-brown pp.
Dark red Austr. bark (probably an *acacia*).	Do.	Pp. needle crystals.	No react.	Deep violet pp.	No react.	Faint trace.	Orange-pink.	Crimson dilutes pink.	Bright violet pp.
"White bark" *Algaroba blanca*. South America (*A. prosopis* or *acacia*).	Do.	Pp.	No react.	Reddish-black pp.	No react.	Violet.	Reddens strongly.	Do.	Red pp. turning. violet.

[1] Used at Cape of Good Hope.

TABLE V

Class 2β	Ferric alum	Bromine water	Nitrous acid	$CuSO_4$ + NH_4OH	$SnCl_2$ + HCl	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
English oak (*Quercus robur*).	Blue-black (green with excess).	Pp.	Reacts somewhat.	Slight pp. Dark brown pp.	No react.	Faint react.	Reddens.	Crimson dilutes pink.	Reddish-brown pp.
Jaft or Dchit.[1] Supposed oak product.[2]	Blue-black pp.	Pp.	Reacts red-blue.	Brown pp. Dark brown pp.	No react. Dark brown pp.	Do.	Some darkening.	Do.	Do.

[1] A Persian product, dark scales very rich in tannin about (40 %).
[2] Strong infusions, dry whitish and iridescent.

TABLE VI

Class 3α	Ferric alum	Bromine water	Nitrous acid	$CuSO_4$ + NH_4OH	$SnCl_2$ + HCl	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
Aleppo galls (of *Quercus infectoria*).	Blue-black pp.	No pp. slight scum.	Reacts red to blue.	Dark pp. insoluble.	Light yellow pp.	No react.	No react.	Greenish to dirty yellow.	Pale pp. turning bluish-green.
[1] **Sumach** (leaf of *Rhus coriaria*).	Do.	No pp.	Reacts feebly.	Dark-brown insoluble pp.	No react.	No react.	No react.	Yellow.	Yellow pp. turning bright green.
[1] **Myrobalans** (*Terminalia chebula*).	Do.	No pp.	Reacts red to blue.	Dark insoluble pp.	No react.	No react.	Yellow.	Yellow.	Yellow pp. turning greenish.

[1] Dyes yellow on Sn. mordants.

TABLE VI.—(*Continued*)

Class 3a	Ferric alum	Bromine water	Nitrous acid	$CuSO_4$ + NH_4OH	$SnCl_2$ + HCl	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
Pomegranate rind (*Punica granatum*).	Blue-black pp.	No pp.	Reacts red to blue.	Dark-brown insol. pp.	No react.	No react.	No react.	Orange-brown.	Bright yellow pp. turning red with excess.
Algarobilla (*Cæsalpinia brevifolia*).	Do.	No pp.	Do.	Dense dark pp.	No react.	No react.	Deep yellow.	Deep yellow-brown.	Bright yellow pp. darkens some.
[1] Divi-divi (*Cæsalpinia coriaria*).	Do.	No pp.	Do.	Do.	No react.	No react.	No react.	Crimson.	Yellow pp. turning red-purple.
Algarobo (*Prosopis dulcis*).	Do.	Do.	Red to olive.	Do.	No react.	No react.	Yellow.	Yellow to olive.	Yellow pp. turning black.
Valonia (*Quercus Aegilops*).	Do.	Do.	Red to blue.	Dark, reddish pp.	No react.	No react.	Purplish-pink.	Deep yellow.	Yellow pp. turning red-purple.
[2] "Oakwood" extract (oak or chestnut).	Do.	Do.	Do.	Purple-brown pp.	No react.	No react.	Reddens.	Yellow-brown.	Do.

[1] Moderately strong potassium nitrate solution precipitates divi-divi, but not *dilute* oak-wood solutions; pp. soluble in hot or ***much*** cold water.
[2] *Crude* chestnut wood extract may be distinguished from oakwood by its violet coloured indication with ammonium sulphide.

TABLE VII

Class 3β	Ferric alum	Bromine water	Nitrous acid	$CuSO_4 + NH_4OH$	$SnCl_2 + HCl$	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime water
Pure gallotannic acid.....	Blue-black pp.	No pp.	No react.	Dark pp.	No react.	No react.	No react.	Yellow.	Pale pp. turning blue.
Babool pods (*Acacia Arabica*).	Blue-black.	No pp.	No react. darkens.	Dark green colour.	No react.	Faint violet.	No react.	Reddish violet.	Pink colour. No pp.

TABLE VIII

Class 3β	Ferric alum	Bromine water	Nitrous acid	$CuSO_4 + NH_4OH$	$SnCl_2 + HCl$	Deal shaving and HCl	Na_2SO_3	H_2SO_4	Lime-water
Catechol................	Dark green colour.	No pp.	Turns yellow.	Green colour.	No react.	No react.	No react.	Green colour.	No react.
Protocatechuic acid.......	Dark green colour.	No pp.	Turns brown.	No pp.	No react.	No react.	No react.	No react.	No react.
Phloroglucinol...........	No react.	Bulky white pp.	Turns olive-green.	No pp.	No react.	Red violet colour.	No react.	Slight yellow.	No react.
Pyrogallol..............	Blue-black turning green and brown.	No pp.	Turns yellow.	Brown colour.	No react.	No react.	No react.	Brown colour.	Violet rapidly turning brown.
Gallic acid..............	Blue-black colour.	No pp.	Turns brown.	Brown colour.	No react.	No react.	No react.	No react.	White pp. rapidly turning blue.

TABLE IX.—REACTIONS OF PURIFIED OAK BARK TANNINS (Trimble, *Tannins*, Vol. 2, p. 88).

	Ferric alum	Bromine water	Nitrous acid	$CuSO_4 + NH_4OH$	$SnCl_2 + HCl$	Deal shavings and HCl	Na_2SO_3	Lime-water
Black oak (*Q. tinctoria*)	Green colour and pp.	Yellow pp.	Brownish-yellow pp.	Pp. Green colour.	Yellow with some pink.	Violet colour.	Yellow colour.	Pp. turning pink then red.
Pin oak (*Q. palustris*)	Green colour and pp.	Yellow pp.	Pinkish colour changing to brown pp.	Pp. Brownish-green colour.	Pink colour.	Violet colour.	Pink colour.	Pp. turning pink then red.
Scarlet oak (*Q. coccinea*)	Bluish-green colour. Green pp.	Yellow pp.	Brown pp.	Pp. Green colour.	Pinkish colour.	Violet colour.	Pinkish yellow colour.	Pp. turning reddish.
Spanish oak (*Q. falcata*)	Green colour and pp.	Yellow pp.	Brown pp.	Pp. Red-brown colour.	Yellow colour some pink.	Violet colour.	Yellow with pink streaks.	Pp. turning reddish.
White oak (*Q. alba*)	Green colour and pp.	Yellow pp.	Brown pp.	Pp. Brown-green colour.	Pinkish colour.	Violet colour.	Pinkish colour.	Pp. turning pink.
Willow oak (*Q. phellos*)	Green colour and pp.	Yellow pp.	Brown pp.	Pp. Red-brown colour.	Very yellow colour.	Violet colour.	Yellow with pink streaks.	Pp. turning green, liquid reddish.
Chestnut oak (*Q. prinus*)	...	Yellow pp.	...	No pp. Greenish-brown colour.	...	...	...	Pp. turning pink.
Swamp white oak (*Q. bicolor*).	...	Yellow pp.	...	...	...	...	...	Pp. turning pink.
English oak (*Q. robur*)	Bluish-green colour. Green pp.	Yellow pp.	Pink colour changing to brown pp.	Pp. Red-brown colour.	Decided pink colour.	Violet colour.	Pink colour.	Pp. turning pink
Indian oak (*Q. semicarpifolia*).	Green colour and pp.	Yellow pp.	Brownish-yellow pp.	...	Pink colour.	Violet colour.	Yellow colour.	Pp. turning pink.

REAGENTS FOR PROCTER'S TESTS

The reagents employed in the above tests are prepared and used as follows:

Ferric alum, a 1% solution. This salt appears to be better than ferric chloride or acetate. It may be reasonably assumed that any tannin which gives a distinctly greenish-black with iron is a catechol derivative, but there are a large number of materials, especially among the acacias or mimosas, which give purplish blacks, but are almost certainly catechol tannins; and, on the other hand, the oak barks which Trimble has proved to be catechol tannins, and most of which give green-blacks, with iron, also yield "bloom," or ellagic acid, and are therefore also allied to gallic acid. Another reagent is therefore employed in the preliminary classification, viz.,

Bromine Water.—It is best to add this drop by drop to 2 or 3 c.c. of the infusion in a test-tube until the solution smells strongly. In some cases the precipitate is slight, or forms slowly, and occasionally it is crystalline, and on this account less easily recognised, but it is usually a distinct yellow or brown flocculent one. In general terms it may be said to be a reagent for the catechol tannins, precipitating all which give green-blacks with iron, and many which give blue or violet-blacks, which are reasonably suspected of containing catechol. It does not precipitate any recognised pyrogallol tannin, but several which yield ellagic acid (bloom), such as oak barks.

Nitrous Acid Reaction.—This reaction is obtained by adding to a few c.c. of the very dilute infusion in a porcelain basin a distinct excess of freshly prepared solution or a few crystals of sodium or potassium nitrite, and then 3–5 drops of N/10 sulphuric or hydrochloric acid. In typical cases the solution instantly turns pink or crimson, and slowly changes through purple to a deep indigo-blue, but in others, as sumach, where the reaction is feeble, and masked by other changes, the final colour is green or even brownish. In a large number of cases, nitrous acid produces a yellow or brown coloration or precipitate, but "reaction" in the tables invariably means a series of colour-changes as above-described. The reaction is given by all tanning materials which yield ellagic acid or "bloom," but not by ellagic acid itself, nor by pure gallotannin. It is therefore probably a reaction of ellagitannin, and is valuable for subdividing the mixed and pyrogallol tannins. It may also be obtained faintly from some of the oaks in Class I.

Copper Sulphate and Ammonia.—A 1% solution of copper sulphate is employed, and is added to the tannin solution first, followed by ammonia in slight excess.

Stannous Chloride and Hydrochloric Acid.—This reagent consists of a strong solution of stannous chloride in concentrated hydrochloric acid. If about 10 c.c. of this are added to 1 c.c. of the tanning material infusion, in a porcelain basin, and allowed to stand for 10 minutes, coniferous tans, mimosas, and some others give a very marked pink coloration. This is specially distinct in the case of larch bark. If a small piece of larch-tanned leather be steeped in the reagent, the coloration appears very strongly.

Deal Shaving and Hydrochloric Acid.—A shaving or slip of any pine wood is moistened with the infusion, and then, either before or after drying, is again moistened with concentrated hydrochloric acid. In the case of cutch and gambier, and a few other materials, and of solution of phloroglucinol itself, the spot becomes at once a bright red or violet, but in many cases the indication is faint, and only appears after some hours. It probably always indicates the presence of phloroglucinol. The statement that catechol gives a similar reaction appears to be incorrect.

Sodium Sulphite.—A few drops of the tannin solution are placed in contact with a crystal of sulphite on a tile. Many tanning materials produce red or pink colorations, but in no case so marked an indication as valonia.

Sulphuric Acid.—About 1 c.c. of concentrated acid is cautiously added to a few drops of the tannin solution in a test-tube so that the acid forms a layer beneath the tannin. The ring of colour at the junction of the 2 liquids is noted, and then they are mixed by shaking and diluted with water.

Lime-water is a most useful reagent. The action is best seen in a shallow porcelain dish, and time must be allowed for the changes to take place.

The infusions of tanning materials employed should contain about 0.6 grm. of dry soluble matter in 100 c.c.

Most tannins possess dyeing properties, giving a variety of brownish or yellowish shades on textiles.

These properties have been recommended by Paessler (*Collegium*, 1906, 287) as a qualitative test for tannins. For that purpose he uses mordanted calico, and reference must be made to Paessler, and

also to Nierenstein (*Chem. Żeit.*, 1906, 32) who has criticised Paessler's test. There seems to be an intimate connection between the colouring principle and the tannin matters contained in the same plants. On decomposition, the same acid, and in some cases the same phenol, is obtained from both. The following table will show the correspondence in the decomposition products of the tannin and its respective colouring matter:

	Tannin	Decomposition product of tannin	Colouring matter	Decomposition product of colouring matter
Quebracho Colorado..	Quebracho-tannin.	Phloroglucinol and protocatechuic acid.	Fisetin.	Resorcinol and protocatechuic acid.
Rhus coriaria........ Rhus cotinus........	Gallotanin.	Gallic acid.	Myricetin.	Phloroglucinol and gallic acid.
Gambier catechu..... Acacia catechu.....	Catechin.	Phloroglucinol and protocatechuic acid.	Quercetin.	Phloroglucinol and protocatechuic acid.
Divi-divi........	Ellagitannin.	....	Ellagic acid.	....

A. G. Perkin (*J. Chem. Soc.*, 1897, **71**, 170) finds that the colouring principle of Cape sumach is a glucoside, osyritrin, $C_{27}H_{30}O_{17}\ 2H_2O$, which is decomposed by acid into quercetin and dextrose. Venetian sumach showed the presence of myricetin, and not quercetin as stated by Lowe.

Valonia, divi-divi, myrobalans, algarobilla, and gall-nuts, owe any dyeing powers to ellagic acid, and contain no member of the quercetin group.

Andreasch (*Gerber*, 1894, **20**, 195 and 207) gives in tabular form the reactions in alcoholic solutions. These have been used by the Vienna Institute for the examination of such extracts obtained from finished leathers.

Detection and Differentiation of Vegetable Tannins.—The detection of adulteration of tannins by cheaper tannins is a matter of great difficulty to the general analyst, or even to the specialist.

Lead Acetate Test.—To 5 c.c. of tannin solution (analytical strength) 5 c.c. of 10% lead acetate solution are added, and a portion of the clear filtrate is mixed with an excess of sodium hydroxide (10%). Mangrove, mimosa, oakwood, chestnut, myrobalans, valonia, divi-divi, algarobilla and gallic acid give colourless solutions. Quebracho and ulmo give a slight but distinct coloration, whilst

Pistacia lentiscus and sumach give a decided yellow, and wood pulp a deep yellow coloration (Stiasny).

Acetic Acid Lead Acetate Test.—This test has been applied quantitatively to the separation of certain tannins. In the following test (Stiasny, *Collegium*, 1912, 483) 5 c.c. of tannin solution (analytical strength) are mixed with 10 c.c. of acetic acid (10%) and 5 c.c. of lead acetate (10%), and the formation of the precipitate of lead tannate observed.

Catechol tannins (quebracho, mangrove, mimosa, ulmo, and gambier) give no precipitate.

Pyrogallol tannins (chestnut, oakwood, myrobalans, sumach, valonia, divi-divi, algarobilla, and gallotannin) give precipitates. (Note: *Pistacia lentiscus* behaves like a pyrogallol tannin in this test.) The precipitate is filtered off, and the clear solution tested with a few drops of ferric alum solution (1%). Quebracho, mangrove, ulmo and gambier give a green colour, whilst mimosa, myrobalans, sumach, divi-divi, algarobilla, and gallotannin give a deep bluish violet. Chestnut gives a very faint violet, whilst oakwood and valonia remain colourless. By this test it is claimed that an addition of 25% of chestnut to an oakwood extract can be detected. In this connection reference must be made to Jean's test for the detection of chestnut extract in oakwood extract (page 47).

Formaldehyde Test.—This test was originally introduced by Stiasny in 1905 (*Der Gerber*, 186) and it stands now in its final form as follows: To 50 c.c. of tannin solution (analytical strength) 5 c.c. of concentrated hydrochloric acid are added and then 10 c.c. of formaldehyde (40%). The mixture is boiled beneath a reflux condenser for 30 minutes, cooled to the ordinary temperature and filtered. The appearance of the precipitate while boiling is noted, and the filtrate used for the following test: To 10 c.c. of the filtrate 1 c.c. of ferric alum solution (1%) is added and about 5 grm. of solid sodium acetate, without shaking. Observe if a blue (or violet) colour appears in the lower layer. Catechol tannins are entirely precipitated by this reagent, the filtrate giving no indication with the iron salt. A portion of the pyrogallol tannins may be precipitated under this treatment, but all of them respond to the test with the iron salt. In practice this test may be used to determine admixture of pyrogallol tannins with catechol tannins. Certain sulphated quebracho extracts (catechol) are not entirely precipitated by this

reagent (Schell). After 30 minutes' boiling in the prescribed manner these special extracts are not entirely precipitated. In this case, however, and even if the filtrate be strongly coloured, it will give no distinct indication, or at least no blue coloration after the addition of the ferric alum solution and sodium acetate (Stiasny). This treatment with formaldehyde does not give a quantitative separation, as a considerable proportion of the pyrogallol tannins are co-precipitated with the catechol tannins. Small (*J. Amer. Leather Chem. Assoc.*, 1911, **6**, 107) has suggested a modified process which Stiasny thinks may give a quantitative separation.

Ware's Modification of Stiasny's Formaldehyde Test.—Stiasny's test has recently been applied in a modified form by A. H. Ware (*Year-Book of Pharmacy*, 1924, 658) to a number of tannin-containing drugs with good results. Ware's procedure is as follows: A few c.cs. of the extract are boiled for about 1 min. with a few drops of a 40% formaldehyde solution and the same number of drops of a 10% solution of dilute hydrochloric acid. The presence of excess of formaldehyde and acid should then be ensured, and the mixture again boiled if necessary. The solution is then cooled and filtered. Either a strong water extract may be used or a water-diluted alcoholic extractive from which most of the alcohol has been boiled off. The latter should not be filtered.

Examination of the Precipitate.—The precipitate should be treated successively, on the paper, with water, 90% alcohol and aqueous alkali. A copious coloured precipitate after this treatment is an almost certain indication of a phlobatannin body. Unless the boiling is much prolonged, gallotannin drugs give little or no residue.

Examination of the Filtrate or Solution.—Dilute, if necessary, and add one or two drops of ferrous sulphate (conveniently kept as 10% ferrous sulphate and 33% cane sugar solution). Then add, drop by drop, a 5% solution of aqueous potash, until either a characteristically coloured solution or a precipitate is produced. Unless the content of the chromogenic phenol is very small or none, there will be no precipitate of the ferroso-ferric hydrate.

It has been noted by Ware that under the conditons of the test, ferric salts often give a green and not a blue coloration, if gallic acid or gallotannin are present. However, if ferrous sulphate is used it can always be relied on to discriminate between iron-blueing and iron-greening substances. In this connection reference must

also be made to the observations of Miss P. H. Price (*Analyst*, 1924, **46**, 27), where it is shown that, whereas ferrous sulphate and chloride detect 0.005% of gallotannin, ferric salts are only able to detect 0.01% of gallotannin.

Ware summarises his results as follows: All phlobatannins yield a characteristic precipitate and gallotannin drugs give an *iron-blueing* filtrate. In addition to the *iron-blueing* filtrate, *iron-greening* and *iron-browning* filtrates are obtained, which are, however, not due to the presence of tannin-like bodies. This was confirmed by Ware by the "Goldbeater's Skin Test" for tannins. (Atkinson and Hazleton, *Biochem. J.*, 1922, **16**, 516; Price, *Analyst*, 1924, **46**, 25.)

THE GOLDBEATER'S SKIN TEST

Tanning consists in the fixation of the tannins by animal fibre, and the goldbeater's skin test demonstrates the specific property of the tannins. The test was elaborated in the writer's laboratory by Atkinson and Hazleton, (*Biochem. J.*, 1922, **16**, 516) and subsequently extended by P. H. Price (*Analyst*, 1924, **46**, 25) in collaboration with R. A. E. Colborn and E. S. Smyth; with the result that it now stands as follows:

General Technique.—A small piece of goldbeater's skin, about 0.5 inch long and 0.75 inch wide, is pinned on a flat surface of paraffin-wax, which is prepared by pouring melted paraffin-wax into a watch-glass.

1. *Swelling.*—One c.c. of 2% solution of hydrochloric acid is pipetted on to the skin and left standing for 10 minutes. The skin is then washed with distilled water at a constant drip of two drops per second for two minutes.

2. *Tanning.*—The skin is treated with 1 c.c. of the solution to be investigated for the presence of tannin for 30 minutes. It is then washed as before for 15 minutes.

3. *Staining.*—One c.c. of a 1% solution of either ferrous sulphate or ferrous chloride is left standing on the skin for 15 minutes and, as before, the skin is washed for two minutes.

4. *Decolourising When Testing for Phlobaphenes.*—One c.c. of a 5% solution of hydrochloric acid is left on the skin for 2 minutes, and then the skin is washed, as before, for 2 minutes.

When dry, the skin may be mounted for reference and compared with skins treated with gallotannin solutions of varying concentra-

tions. When very minute quantities indeed of tannins are suspected to be present, it is advisable to compare the skin also with untanned stained skins and stained skins which have been treated with gallic acid.

C. J. Jordan and A. H. Ware (*Year-Book of Pharmacy*, 1924, 651) have used this test with satisfactory results and A. H. Ware (*ibid.*, 660) has summarised the advantages of the Goldbeater's Skin Test over his modifications of Stiasny's Formaldehyde Test as follows: The Goldbeater's Skin Test has the following advantages, namely that it distinguishes between gallic acid and gallotannin, and also between iron-greening anthoxanthins which stain the skin and such iron-greening bodies as chlorogenic and ipecacuanhic acids which do not stain the skin.

Ware's Tests.—In a series of publications which have appeared in the *Analyst* and the *Pharm. J.* during 1924 and 1925 A. H. Ware has described a number of qualitative tests which are given in the following tables:

1. Tests Depending upon the Precipitation of Ferroso-ferric Tannates from Iron Complexes.—If tannins, in solutions or extractives, are boiled with the citrate of iron and ammonia of the British Pharmacopœia, coloured iron complexes are formed which are held up in solution. From such a solution, by the use of a suitable salt, the ferroso-ferric tannate may be thrown out as a coloured precipitate. Different classes of tannins may be distinguished by appropriately varying the salt and the details of method used. The methods used may be briefly summarised thus:

1. 0.5 grm. of Na_2SO_3 (to prevent undue premature precipitation of tannin) and 1 grm. of $NH_4\bar{A}$, both in small crystals, are boiled with 5 c.c. of fresh aqueous or water diluted (unfiltered) commercial extractive. The mixture is filtered, if necessary, and the precipitate neglected. It is then boiled with 5 c.c., or more, of the ferric citrate solution (0.25% aq.) and again filtered.

A purple or violet ppt. indicates tannin. (In the case of logwood the violet or blue ppt., is due to the tannin together with partially precipitated hæmatoxylin.) If the ppt. be brown it may be due either to certain phlobatannins or to partially precipitated phloroglucinol-catechol flavones or flavonols. It will then be necessary to try method (2) in order to distinguish between these. In the presence of tannin, a blue, violet, or purple filtrate generally indicates either

hæmatoxylin, gallic acid, catechin or, possibly, brazilin. In the absence of a final ppt., a purple or violet solution is most likely to be due to either ipecacuanhic acid, brazilin, so-called tobacco-tannin or chlorogenic (caffetannic) acid.

2. The extractive is boiled *first* with the iron reagent, filtered if necessary, and then boiled with 1 grm. of NH_4Cl. All tannins are more or less completely precipitated (in logwood, also the hæmatoxylin partially) but anthoxanthins are not. Should the result be negative with respect to tannins, the production of a brown ppt., if considerable, on adding and boiling with, $NH_4\bar{A}$ and NH_3 aq., shows the presence in the extractive of a flavone or flavonol of the type referred to under (1).

3. Typical pyrogallo-tannins (gallotannins and ellagitannins) as a class, may be readily distinguished from phlobatannins, by adding *first* to the extractive 10–12 drops of acetic acid (30%) and then proceeding as in (2). Gallotannins, ellagitannins and hæmatoxylin (partially) are precipitated but phlobatannins are not.

4. A method which may give less complete precipitation than *either* of the other methods described, but goes *much further* in the direction of distinguishing between different classes of tannins, is as follows, viz:—*First* boil 1.5 grm. of sodium dihydrogen phosphate with the extractive. Filter, if necessary, and then boil with the ferric citrate solution. True gallotannins are partly or completely precipitated as violet iron-compounds, ellagitannins as green-black compounds, whilst tannins of hamamelitannin type are usually not precipitated but give a deep-brown solution. Should tannins of the last class be precipitated, the iron compound is always definitely brown. This method is also very useful in distinguishing hæmatoxylin. Neither this body nor the tannin associated with it in logwood is precipitated by this method, but a brown solution is given. This suggests a relationship between these bodies and hamamelitannin. Phlobatannins are also not precipitated, but these are best distinguished by method (3).

II. Modification of Mitchell's Ferrous Tartrate Test.—The extractive or solution is made with distilled water to which 10–12 drops of acetic acid (30%) has been added. Mitchell's reagent made with distilled water is then added, and the mixture warmed. A blue or violet solution is given only in the case of the presence of

true gallotannins or anthocyanins. Ellagitannins often give a green colour.

III. Modifications of Procter's Test for Ellagitannins.—Extractives containing ellagitannins will all give a green colour-reaction if warmed cautiously with a few crystals of $NaNO_2$. If NaH_2PO_4 be first added and very little $NaNO_2$ be used, a deep blue colour may be given instead of a green. If 3 or 4 drops of 10% acetic acid be also added before warming, a rich violet colour is often given. No other plant-phenols are known to give similar colour-reactions under the same conditions.

IV. Test with Iodine and Ammonia.—Phlobatannins are precipitated from water solution by boiling with a few drops of tincture of iodine. Most other plant-phenols, including typical pyrogallol-tannins, are not so precipitated. The presence of such phenolic substances in the filtrate is readily indicated by adding NH_3 aq., when an intensification of colour occurs. Phlobatannins differ with respect to the character of the ppt. given, for this is in some cases soluble in solution of ammonia and in others insoluble. This distinction affords a useful method of classifying phlobatannins in conjunction with other characters (*Ph. J.*, July 26, 1924).

V. Modification of Stiasny's Test.—This modification is described on page 77.

VI. Tests for Aromadendrin and Kino-yellow.—A strong decoction or thin paste is skaken out with ether. The ethereal layer is separated and the ether allowed to evaporate. The residue is dissolved in a few c.cs. of 90% alcohol, a piece of pure zinc added together with 12 drops of HCl (conc.) and the mixture warmed. A rich pink to a cerise-red colour is produced if any appreciable quantity of aromadendrin is present. The reaction differs from that given by flavonols as the colour is destroyed by a slight excess of alkali without any characteristic colour replacing the red. In kinos aromadendrin is generally accompanied if present in marked amount by kino-yellow. The presence of kino-yellow can be indicated by evaporating an alcoholic extractive with a few drops of dilute H_2SO_4 when a yellow film will be shown on the side of the dish, if the latter be occasionally agitated.

TABLE I.—FOR DETERMINING THE PRESENCE OF TANNIN AND FOR ASSIGNING SUBSTANCES CONTAINING TANNIN TO CERTAIN PRIMARY CLASSES

<table>
<tr><td colspan="6">First apply the ferric citrate test in the presence of Na_2SO_3 and $NH_4\bar{A}$ (I. 1 page 79).</td></tr>
<tr><td colspan="5">A ppt. is given.</td><td>No ppt. is given.</td></tr>
<tr><td colspan="5">Filter and note the nature of the ppt. and filtrate.</td><td rowspan="11">Tannins are absent. Quercetin and luteolin are also probably absent.</td></tr>
<tr><td colspan="4">Ppt.</td><td>Filtrate.</td></tr>
<tr><td colspan="4">If blue, violet or purple the next step may be omitted, and the presence of tannin assumed.</td><td rowspan="9">If blue violet or purple, hæmatoxylin (in logwood), gallic acid or catechin, are most probably present. Among phlobatannin substances, note particularly the bark of Acacia decurrens for gallic acid, and acacia cutches and guarana for catechins.</td></tr>
<tr><td colspan="4">If the ppt. is brown boil a fresh portion of the extractive with the ferric citrate solution, cool and filter. Add NH_4Cl and again boil.</td></tr>
<tr><td colspan="3">A ppt. is given. Tannin is present.</td><td>No ppt. is given. Tannin is absent.</td></tr>
<tr><td colspan="3">Repeat the last test, adding acetic acid before the other reagents.</td><td rowspan="6">Add $NH_4\bar{A}$ and NH_3 aq. and again boil. A brown ppt. indicates the presence, probably of luteolin or a quercetin glucoside. The extractive should give a green colour reaction to ferric alum and no typical ppt. for tannin to the formaldehyde test.</td></tr>
<tr><td>No ppt. is given.</td><td colspan="2">A ppt. is given.</td></tr>
<tr><td rowspan="3">Absence of typical pyrogallol-tannin may be assumed. Add $NH_4\bar{A}$ and NH_3 aq. A ppt. should now be given. Confirm phlobatannin by Stiasny's and Iodine tests and note results for future use.</td><td colspan="2">Apply the formaldehyde and HCl test to a fresh portion of the extractive.</td></tr>
<tr><td>Typical ppt. given.</td><td>No typical ppt. given.</td></tr>
<tr><td>The substance contains both phlobatannin and typical pyrogallol-tannin. The filtrate from the formaldehyde test will with rare exceptions give a violet if tested with iron as directed.</td><td>Phlobatannin is absent. Pyrogallol-tannin is present. Confirm by Stiasny's, Br. water and I. tests applied to fresh aq. ext., or to a commercial ext. well diluted with water and filtered.</td></tr>
<tr><td>See Table II.</td><td>See Table III.</td><td>See Table IV.</td></tr>
</table>

TABLE II.—FOR THE SEPARATION OF TYPICAL PHLOBATANNIN SUBSTANCES

Typical Phlobatannin Substances will possess the following characters, which will distinguish them readily from Typical Pyrogallol-tannin Substances. An extractive boiled with the ferric citrate reagent and acetic acid, cooled and filtered, gives no ppt. if reboiled with the addition of NH_4Cl. On the contrary a very characteristic ppt. is given if an extractive is boiled with formaldehyde and HCl. The extractive will give a ppt. with bromine water in the cold and with iodine on boiling and cooling.

They may be subdivided primarily by considering the results of applying the following tests, viz. (1) The addition of ferric alum, 1 %, to a clear dilute aqueous or alcoholic extractive, (2) Applying Stiasny's test and testing the filtrate for iron-blueing properties, and (3) Adding to the watery extractive 1 % $CuSO_4$ aq., and then NH_3 aq. in excess. According to the results given they fall into two main classes, thus:

Either ferric alum gives with the extractive a blue, violet or purple colour-reaction or the filtrate from Stiasny's test gives one of these colours on adding $FeSO_4$ and sufficient 5 % aq. KOH. The ppt. given by $CuSO_4$ and NH_3 is almost always insoluble in excess of NH_3 aq. See Table II*A*.	Ferric alum always gives a green, olive-green or brownish-green. The filtrate from Stiasny's test never gives a blue, violet or purple colour-reaction with $FeSO_4$ but may occasionally give a green solution due to the presence of an anthoxanthin. The ppt. with $CuSO_4$ and NH_3 aq. may or may not be soluble in excess of NH_3 aq. See Table II*B*.

TABLE II*A*

Boil the fresh aqueous extractive or water-diluted and filtered commercial extractive with a slight excess of tincture of iodine. Cool and filter. Test the solubility of the ppt. in NH_3 aq. (10 %).

The ppt. given is either quite insol. or mostly insol. in NH_3 aq. All the bodies at present included in this section form a very natural class in other respects. See Table V. Section 1.	The ppt. given on boiling with iodine is either quite soluble or very large y soluble in NH_3 aq. These can be best subdivided at first by boiling a little of the extractive in a dish with a few drops of dilute H_2SO_4. Try first a watery extractive and if that gives no very distinctive result then try a 60 % alcoholic extractive. The dish should be agitated occasionally.		
	Yellow film given in alcoholic extract.	Crimson-purple film given in aq. extract.	Rose-purple film given in aq. or alcohol-extract.
	Apply test for aromadendrin. If a positive result is given: See Table V. Section 2.	Ferric alum gives a blue purple or violet. $CuSO_4$ and NH_3 ppt. always insol. in excess of NH_3. See Table V. Section 3.	Ferric alum reaction is predominantly brown. Apply special test for emodins. See Table V. Section 4.

TABLE II*B*

These are best subdivided primarily by considering the behaviour of the ppt. given to the iodine test, and that given to the $CuSO_4$ and NH_4 test, each respectively, with excess of NH_3 aq.

The ppt. with iodine is sol. or very largely so. The ppt. given to the $CuSO_4$ test is also soluble in excess of NH_3 aq.		Ppt. with I as last, but that with $CuSO_4$ is insol., in excess of NH_3.	Ppt. with I insol. or mainly so. Ppt. with $CuSO_4$ etc. also insol.	Ppt. with Iodine is insol. but ppt. with $CuSO_4$ etc., is sol. in excess of NH_3 aq.
Apply the deal shaving and HCl test.		See Table V. Section 9.	See Table V. Section 10.	See Table V. Section 11.
Magenta or violet stain.	No characteristic stain.			
See Table V. Section 5.	See Table V. Sections 6, 7, and 8.[1]			

[1] Sections 6, 7, and 8 are well distinguished from one another, but are grouped together in the adjoining column to avoid getting a multiplicity of very small tabular subdivisions.

TABLE III

For separation of substances containing both phlobatannin and typical pyrogallo-tannin.

These are well distinguished as a class by giving a ppt. in extractive to the ferric citrate and ammonium chloride test in the *presence* of acetic acid, and by also giving a characteristic ppt. to the modified Stiasny's test. They probably all give an iron-blueing filtrate to Stiasny's test. They usually give a ppt. with $CuSO_4$ and NH_3 which is insol. in excess of NH_3. They give a ppt. to the Br. water and iodine tests respectively. If a little of the extractive be mixed with a few crystals of $NaNO_2$ on a tile and a little 10% NH_3 aq. be placed on the tile near by, and be caused to flow slowly into the extractive by tipping the tile, a pink colour will nearly always be given which often becomes fringed with green. They may be subdivided primarily by the iodine and ammonia test.

Ppt. with Iodine completely sol. in solution of NH_3	Ppt. with iodine incompletely soluble in solution of NH_3.
See Table V. Section 12.	See Table V. Section 13.

TABLE IV

For the separation of substances containing typical pyrogallol-tannins, but no phlobatannins into subclasses.

The class as a whole is well-defined by the possession of the following characters, viz.—The tannins may be precipitated from their extractives by adding acetic acid and the ferric citrate reagent, boiling and filtering, and then adding to the filtrate NH_4Cl and reboiling. On the contrary the tannins are not appreciably precipitated by the formaldehyde and HCl test. With rare exception (see Kousso) a dilute extractive gives a blue, purple or violet colour-reaction with 1% ferric alum. They give a ppt. with $CuSO_4$ and NH_3 which is insoluble in excess of NH_3 aq. In clear aqueous extractive or clear diluted and filtered commercial extractive they give no ppt. in the cold with Br. water or on boiling with iodine. The extractives give characteristic colour reactions with lime water, also on a tile with ammonia (see Table III). They can be subdivided as follows:

Tests employed.	Contain gallotannin but little or no ellagitannin.	Contain ellagitannin but little or no gallotannin.	Contain both gallotannin and ellagitannin.	Contain tannin of the type of Hamamelitannin, but little or no gallotannin or ellagitannin.	Contain tannin of dubious class.
Mitchell's reagent in the presence of HA (see test II, p. 80).	Definite blue or violet solution always given.	No definitely blue or violet colour given but sometimes green.	As in col. 2, *i. e.* section 1.	As in col. 3, *i. e.*, no blue or violet.	Dubious result.
Apply test I (4), *i. e.*, the ferric citrate test in presence of NaH_2PO_4.	A violet or blue ppt. or occasionally a deep violet solution is given.	A green-black ppt. is usually given.	As in col. 2, *i. e.*, section 1.	Usually no ppt. is given but a deep brown solution. If any ppt. it is brown.	Purplish or brown ppt. given.
Apply tests III, *i. e.*, with $NaNO_2$ alone or with acid.	Brown solution only given.	Green, blue violet or purple solutions given.	As in col. 3, *i. e.* section 2.	As in col. 2, *i. e.* section 1.	As in col. 2, *i. e.* section 1.
Test with lime water.	Usually blue or green ppt. A red ppt. is rarely if ever given.	The ppt. is often red and never blue or violet.	Varies.	Varies but is rarely either red or blue.	Varies.
Stir into extractive on tile $NaNO_2$ and afterwards 1 drop of 10% NH_3 aq.	A green colour is usually developed on standing a short time.	A green colour is seldom given, but there are important exceptions.	A green colour may or may not be given.	As in last col. (4).	As in col. 4.
	See Table VI. Section 1.	See Table VI. Section 2.	See Table IV. A, below.	See Table VI. Section 6.	See Table VI. Section 7.

TABLE IVA

For further subdivision of the subclass of body which contains both true gallotannin and ellagitannin (col. 4 above).
This can be effected by considering the results of the tests with lime water and with sodium nitrite and ammonia (1 drop stirred in). Allow to stand a short time in both cases, if necessary.

Test	Gallotannin predominates	Ellagitannin predominates	Intermediate class
Add the watery extractive gradually to lime water in a white dish, and stir.	The colour is blue, green or brown but never a decided red.	The colour is a characteristic and usually decided red.	The colour is red.
Test with nitrite and NH_3.	A green colour is developed. See Table VI. Section 3.	Little or no green colour given. See Table VI. Section 4.	A green colour is given. See Table VI. Section 5.

TABLE V.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES II AND III

Before using this table, Tables II and III must be consulted, since the characters common to the individual substances belonging to any particular section are not usually repeated in the present table.

No. of section and name of substance	Colour of film given by evaporating ext. with dil. sulph. acid	Reaction with lime water	Effect of ammonia on the filtrate obtained by boiling the ext. with iodine	Test on tile with sod. nitrite or sod. sulphite alone or when so stated with a trace of ammonia	Test on tile with excess of ammonia in the presence of sod. sulphite or sodium nitrite	Special tests, characters or remarks. (For test for aromadendrin see test VI, p. 81)
Section 1. Salix viminalis type.						
Salix viminalis bark.........	Poor purplish film.	Yellow to brown ppt.	Little or no intens. of colour.	Nil distinctive.	Nil distinctive.	Markedly iron-blueing.
Kino from *Eucalyptus sidersphloid.*	Nil distinctive.	Bronze-brown ppt.	Shows incomplete pption.	Nil distinctive.	Yellow in ammonia zone.	Iron-purpling. Contains a trace of aromadendrin.*
K. from E. conacea.........	Do.	Do.	Little or no intensification of colour.	Sod. nitrit. slight purplish-violet.	The sulphite gives a yellow.	Iron-purpling. Distinct trace of aromadendrin.†
K. from E. piperita.........	Do.	Do.	As K. from E. conacea.	Sodium nitrite and a trace of ammonia give a marked purplish-violet.	....	Violet with ferric alum. A small trace of aromadendrin present.‡
Section 2. Kinos with aromadendrin and kino-yellow.						
Kino from *Eucalyptus calophylla.*	Yellow then brown (with an alcoholic extractive).	Brown ppt.	Intensification of colour given.	Sodium sulphite gives reddish colour.	Dull purplish pink.	Ppt. with Stiasny's test not gelatinous: the filtrate is iron blueing, so is orig. extractive.§
Kino from *E. viminalis*......	Yellow then purplish-brown, (with an alcoholic extractive).	Do.	Intensn. of colour given.	Nil distinctive.	Nil distinctive.	Stiasny's test gives a gelatinous ppt. The filtrate is iron-blueing. The extractive is iron-greening.

Kino from *Angophora intermedia*.	Yellow followed by a dull dubious violet in alcoh. ext.	Brown becoming reddish.	Intensn. of colour given.	....	Yellow at junction of fluids pinkish-purple at back.	Gives some positive response to the deal shaving and acid test.
The kinos from *E. Gunnii* and *E. leucoxylon* resemble the above in many respects and certain other kinos to a less degree (see col. 7).	....	....	....	....	....	Many kinos contain both aromadendrin and kino-yellow in considerable quantity (refer to sect. 6 of present table and sects. 5 and 7 of Tab. VI).
Section 3. Mimosa-Tannin Substance						
Acacia decurrens bark.....	Beautiful but evanescent crimson-purple in aqueous ext.	A distinctive whitish violet ppt. changing (in excess of l. water) to a brown.	Much intensifcn. of colour given.	Sod. nitrite and trace of ammonia purplish-brown.	With sodium sulphite, a deep-yellow followed by a brilliant pink.	Filtrate from Stiasny's test is iron-blueing, due to gallic acid, which also probably causes the pink colour-reaction given by the ammonia and sod. sulphite and the violet with lime water.
Acacia pycnanthia bark......	Similar to A. decurrens bark.	Brown ppt. at once.	A little intensification of colour.	Evanesnt. or dirty violet.	Deep-yellow where the ammonia is in excess and purplish at the edges of this.	Differs also from decurrens bark in that the filtrate from Stiasny's test is not iron-blueing and that the iodine ppt. is entirely and not merely partially sol. in aq ammonia.¶
"Mimosa" bark (from an unknown species).	Similar to A. decurrens bark.	Brown at once.	....	Good pink with sod. sulphite.	Deep-yellow in ammonia.	Differs from the other two barks in this section in giving no reaction with the deal shaving and acid test. The extract gives a purple and "Stiasny's" filtrate nil with iron.

* Ppt. given by Stiasny's test is gelatinous.
† "Stiasny's" ppt. is not gelatinous.
‡ "Stiasny's" ppt. is not gelatinous.
§ Ppt. with $CuSO_4 + NH_3$ is sol in excess of aqueous NH_3. Gives good positive response to deal shaving HCL test.
¶ Both these barks give a definitely positive reaction to the deal shaving and hydrochloric acid test.

TABLE V.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES II AND III.—(*Continued*)

No. of section and name of substance	Colour of film given by evaporating ext. with dil. sulph. acid	Reaction with lime water	Effect of ammonia on the filtrate obtained by boiling the ext. with iodine	Test on tile with sod. nitrite or sod. sulphite alone or when so stated with a trace of ammonia	Test on tile with excess of ammonia in the presence of sod. sulphite or sodium nitrite	Special tests, characters or remarks. (For test for aromadendrin see test VI, p. 81)
Section 4. Emodin body. **Chinese** Rhubarb..........	Rose-purple film.	Reddish or bronze-brown ppt.	A great intensification of colour given.	Nil distinctive with sod. nitrite but reddens with the sulph.	Nil distinctive.	With care the filtrate from Stiasny's test will give an iron-blueing reaction. If an extract, is shaken out with petroleum ether and the ethereal layer removed and shaken with aqueous ammonia a rich rose-red colour is given to the latter.
Section 5. Typical Catechu-tannin bodies. **Cube Gambier (the pale catechu of the pharmacist).**	An alcoholic ext. gives a yellow film.	No ppt.	Intensn. of colour given.	Nil distinctive.	Nil distinctive.	Filtrate from Stiasny's test is iron-greening. Gambier contains chlorophyll which may be dissolved out by chloroform (R. P. Biggs). Gambier is best distinguished from cutches by shaking out a strongly alkaline sol. with petrol. ether. The latter will acquire a green fluorescence.

Acacia catechu heart-wood and Acacia cutches (black catechu of the pharmacist).	Slight rose before giving a brown.	Reddish ppt. falls slowly.	Intensification of colour given.	Na_2SO_3 gives yellow.	Yellow to orange.	Distinguished readily from certain substitutes by responding distinctly to the aromadendrin test. (It has not been proved, however, that the body giving this positive response is aromadendrin).
Guarana....................	Nil characteristic.	Slight or no ppt.	As gambier.	Nil distinctive.	Nil distinctive.	After freeing from tannins, etc. by the usual lead and hydrogen sulphide methods, the crude purin bases contained in this body may be obtained and will give the usual reactions (see tea).
Section 6. More Kinos containing Aromadendrin and Kino-Yellow.						
Kino from *E. leucoxylon*.....	Good yellow film precedes purplish-brown.	Brown ppt.	Intensification of colour given.	Nil distinctive.	May give a very slight pink.	Readily distinguished from all similar substances by yielding a purplish-violet filtrate to the ferric citrate precipitation test which is *not* due to either catechin or gallic acid. Contains aromadendrin.
The Kinos from *E. Gunnii* and *E. punctata* have many characters in common with that from E. leucoxylon.	....	....	....	....	....	For the K. from E. gunnii refer to Table III, Sect. 12. For that from E. punctata refer to sect. II of the present table. Both contain aromadendrin and kino-yellow (see test on page 81).

TABLE V.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES II AND III.—(*Continued*)

No. of section and name of substance	Colour of film given by evaporating ext. with dil. sulph. acid	Reaction with lime water	Effect of ammonia on the filtrate obtained by boiling the ext. with iodine	Test on tile with sod. nitrite or sod. sulphite alone or when so stated with a trace of ammonia	Test on tile with excess of ammonia in the presence of sod. sulphite or sodium nitrite	Special tests, characters or remarks. (For test for aromadendrin see test VI, p. 81)
Section 7. Quebracho-Tannin Body.						
Quebracho wood (from *Quebracho Lorentzii*).	Beautiful crimson purple film preceded by a slight yellow.	Reddish to brown ppt.	Some intensification of colour.	No very distinctive reaction.	With sod. nitrite gives a yellow in front of junction of liquids and deep pink behind.	Contains fisetin, hence the filtrate from Stiasny's test is iron-greening.
Section 8. Krameria Type. Krameria or Rhatany root from *Krameria triandra*.	Nil distinctive.	Red ppt. falls slowly.	No intensification of colour.	Red colour given.	Nil distinctive.	Iodine ppt. is completely sol. in aqueous ammonia.
Hemlock bark from *Abies canadensis*.	Dull violet preceding brown.	Reddish brown ppt.	Slight colour on adding ammonia.	Reddish colour (slight).	Nil distinctive.	Readily distinguished from Krameria, etc., by not giving a definitely green colour-reaction with iron and by the incomplete solubility of the Iodine ppt. in aq. ammonia.
Elm bark from *Ulmus campestris*.	Dull purplish.	Yellow ppt. darkening on standing.	A little colour is given to the aq. ammonia.	Nil distinctive.	Pale yellow given in presence of the sulphite.	Slight blackish ppt. given to the copper sulphate and ammonia apparently not quite sol. in excess of the latter. Also readily distinguished from the other bodies at present included in this section by the less intense colour.

Section 9. Mangrove Type. Red Gum from an unnamed species of *Eucalyptus? rostrata*.	Poor purplish.	Brown ppt. at once.	Pption. is incomplete hence a brown colour is always given.	Slight pink colour given.	Nil distinctive.	This was a first-class soluble pharmaceutical variety. Contains a marked trace of aromadendrin.
Ulmus campestris bark	. . .	. . .	. . .	. . .	. . .	See section 8.
Mangrove bark (tanning material).	Poor purplish.	Pinkish colour to red ppt. darkening.	Completely, ppted., hence filtrate gives no colour to the test.	Reddish colour given.	Nil distinctive.	Extractive yields an exceptionally rich red if boiled with NH_3 aq. and cooled. Olive-green only to ferric alum.
Cocoa seeds (*Theobroma cacao*).	Poor purplish.	Brown ppt.	Brown colour given.	Nil distinctive.	Nil distinctive.	Seeds contain much fat. Test for purin bases (see "Tea").
Kino from E. viminalis	. . .	. . .	. . .	. . .	. . .	See section 2.
Section 10. Malabar Kino Type.						
Kino from *Pterocarpus marsupium*.	Definite purplish-violet.	Brown ppt. at once.	Pption. complete hence no colour left on adding NH_3.	Nil distinctive.	Nil distinctive.	Iodine ppt. given *in the cold* is soluble in NH_3 aq. (see Butea gum).
Butea Gum Kino from *Butea frondosa*.	Nil distinctive.	Very pale pinkish-brown.	No intensification of colour.	Slight pinkish colour.	Nil distinctive.	Distinguished from all other tannin bodies tested, by the insolubility of the ppt. given by iodine in the cold (*i. e.*, without the usual boiling) in NH_3 aq.
Prunus serotina bark	Poor purplish.	Brown ppt.	Brown colour left.	Nil distinctive.	Nil distinctive.	Contains quercemeritrin hence the filtrate from Stiasny's test is iron-greening. Also it yields B. Methylæsculetin on boiling with H_2SO_4 (dil). If the acid sol. be shaken out with $CHCl_3$ and the $CHCl_3$ layer be then shaken out with ammoniacal water a greenish-blue fluorescence will be given.
The bark of *Cinnamomum zeylanicum*.	Good purple film.	Pinkish-brown ppt.	No colour left.	Nil distinctive.	Nil distinctive.	The characteristic odour and taste is well-known.

TABLE V.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES II AND III.—(*Continued*)

No. of section and name of substance	Colour of film given by evaporating ext. with dil. sulph. acid	Reaction with lime water	Effect of ammonia on the filtrate obtained by boiling the ext. with iodine	Test on tile with sod. nitrite or sod. sulphite alone or when so stated with a trace of ammonia	Test on tile with excess of ammonia in the presence of sod. sulphite or sodium nitrite	Special tests, characters or remarks. (For test for aromadendrin see test VI, p. 81)
The bark of *Salix fragilis*....	Poor purplish.	Yellow ppt. darkening.	A little colour is left.	Nil distinctive.	Yellow darkening.	Distinguished from the golden osier by the copious ppt. given to the $CuSO_4$ and NH_3 test. It is the chief commercial source of salicin (see S. alba bark).
Section 11. Filicitannin Type.						
Bark of *Larix european*.....	Poor purplish.	Brown ppt.	Some colour left.	Nil distinctive.	A slight pink to $NaNO_2$ and NH_3.	Readily recognised by the characteristic odour, red colour and slightly bitter taste of the alcoholic extractive.
Krameria argentea root......	Dull violet.	Red ppt.	No coloration.	Do.	Little change.	Resembles *K. triandra* except in insoly. of I. ppt. in NH_3 aq.
Kino from *E. punctata*	Yellow film given in alcoholic extract.	Brown ppt.	A little colour remains.	Dull purplish.	Nil distinctive.	Iodine ppt. is partly (lesser part) sol. in NH_3 aq. Gives a very definite reaction for aromadendrin.
Kola Nuts (Dried seeds of *Cola vera*).	Poor purplish-red.	Yellow ppt. becoming yellowish-brown.	Little or no colour given.	Nil distinctive.	Nil distinctive.	Contains much starch. Also contains purin bases (see "Tea").
Bark of *Salix alba*. (Golden Osier variety.)	Poor purplish.	Yellow darkening.	Little or no colour.	Nil distinctive.	Yellow on standing.	By ppting tannins, etc. with PbA_2, adding H_2SO_4 to the filtrate, filtering off $PbSO_4$, neutralising and concentrating, crude salicin may be obtained, giving the characteristic red colour with H_2SO_4.-$CuSO_4$ and NH_3 test

Male Fern Rhizome	Poor violet.	Little if any ppt.	No colour given.	Na_2SO_3 alone pinkish.	Yellowish colour.	Filtrate from Stiasny's gives a slight purple to the $FeSO_4$ and KOH test.
Areca Nut from *Areca catechu*	Definite but dull purplish red	Nil distinctive.	No colour given.	Na_2SO_3 increases pink tint of extractive.	No yellow given.	Yields a red extractive becoming an intense red with NH_3.
***Cinchona succirubra* bark**....	Nil characteristic.	Reddish-brown ppt.	No colour given.	Na_2SO_3 gives a reddish colour.	Nil distinctive.	An alcoholic extractive if freed from alcohol will yield its alkaloids to $CHCl_3$ and NH_3. The $CHCl_3$ layer evaporated leaves a residue which will give the characteristic reactions of quinine.
Hemlock **bark** (*Abies canadensis*).	Poor violet.	Reddish-brown ppt.	A little colour given.	Slight or no reaction.	Slight intensification of colour.	Rich reddish ext. gives little or no green to ferric alum.
Bark of *Cinnamomum Oliveri*.	Poor purplish-violet.	Brown becoming reddish-brown.	Little or no colour given.	Nil distinctive.	Nil distinctive.	Very distinctive odour (partly due to safrol.)
Bark of *Cinnamomum cassia*.	Poor violet.	Brown ppt. falls slowly.	As above.	As above.	As above.	Contains plenty of starch and very little soluble tannin. Characteristic odour resembling but differing from cinnamon.
Section 12. "Tea" Leaves Type. (See Table III.) (N. B. ext. is iron-greening in substances at present included).						
"**Tea**" **leaves (Assam.)**......	Very slight yellow followed by purplish-brown.	Greenish-brown colour followed by a golden-brown ppt.	Some brown given.	Nil distinctive.	Some pink given.	Stiasny's filtrate gives a blue-green reaction to the $FeSO_4$ and KOH test. Crude caffeine giving well-known reactions may readily be obtained by treating a decoction with $Pb\bar{A}_2$ removing lead from filtrate with H_2S and evaporating sufficiently.
Kino from *E. Gunnii*........	Deep yellow followed by mahogany-brown.	Dirty-brown ppt. at once.	Deep-red colour given.	Na_2SO_3 alone a slight purplish-red.	Slight purplish-red.	Gives a very definite reaction for aromadendrin: also well distinguished by giving a ppt. with $CuSO_4$ and NH_3 which is *sol.* in excess of NH_3.

TABLE V.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES II AND III.—(*Continued*)

No. of section and name of substance	Colour of film given by evaporating ext. with dil. sulph. acid	Reaction with lime water	Effect of ammonia on the filtrate obtained by boiling the ext. with iodine	Test on tile with sod. nitrite or sod. sulphite alone or when so stated with a trace of ammonia	Test on tile with excess of ammonia in the presence of sod. sulphite or sodium nitrite	Special tests, characters or remarks. (For test for aromadendrin see test VI, p. 81)
Section 13. Quercitannin Type). (N. B. Extractive usually gives purple or violet with Ferric Alum. Quercus an exception.)						
Quercus robur bark	Slight purpling.	Reddish-brown ppt.	Some brown colour given.	Reddens with Na_2SO_3 alone.	A poor pink given.	Blueish-green colour-reaction with ferric alum.
Bark of *Acacia arabica*	Poor purple.	Dirty brown becoming reddish-brown on standing.	Some colour given.	Nil distinctive.	Good pink given.	Contains but little gallic acid (see A. decurrens bark. Section 3).
Pimento berries (*Pimenta officinalis*).	Good salmon-pink in aq. extractive.	Green, yellowish-to reddish-brown, finally brown.	Colour given.	Little reaction.	Poor pink and greenish colours given to excess of NH_3 but with $NaNO_2$ and 1 drop 10% NH_3 aq. a distinct but transient green.	See Table VI, Section 3, as these berries also appear to contain gallotannin and ellagitannin.
Kino from *E. phellandra*	Nil distinctive.	Bronze-brown ppt.	Little colour given.	$NaNO_2$ alone, a poor violet purple and Na_2SO_3 alone a purplish-pink.	Nil distinctive.	Practically no aromadendrin. Sample examined contained very little water-soluble tannin of any sort, hence the almost negative reaction for gallotannin given by the I test filtrate. The filtrate from Stiasny's test also gives only a poor iron-purpling.

N. B. The small amount of soluble tannin present in many of the kinos appears to be due to the conversion of much of the tannin into kino-red, a change due largely to the presence of oxydases. This probably also accounts for the gelatinous ppts. so often yielded to Stiasny's test.

TABLE VI.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES IV AND IV.1

No. of section and name of substance	Colour of film given by the dil. H_2SO_4 evap. test	Reaction with lime water	Extractive tested on tile with $NaNO_2$ and 1 drop of 10% NH_3 aq.	As last but with excess of NH_3 aq. run in	Special tests, characters or remarks
Section 1. Typical Gallotannin Bodies, with no or very little Ellagitannin.					
Commercial Tannin (or Tannic acid).	Nil in water sol.	Blueish-violet becoming dirty brown-violet.	Green colour-reaction.	Pink, yellow and green colours given.	Commercial tannins often contain gallic acid in which case they will not be completely precipitated in the ferric citrate test.
Chinese Galls..............	Do.	Blue becoming dirty blue.	Do.	Do.	Violet-purple filtrate ferric citrate test due to gallic acid.
Sumach (leaf of *Rhus coriaria*).	Yellow (good).	Yellow green and brownish green colours given successively.	Do.	Marked yellow followed by pink and green.	Na_2SO_3 alone gives a yellow. An alcoholic extract, diluted with water, evaporated and shaken out with ether yields to the ether gallic acid and myricetin.
Red Rose (*Rosa gallica*) petals.	Good yellow, even with aq. extract.	Decided green rapidly changing to brown.	Do.	Green, yellow and poor pink.	The apparent indication of gallotannin given by Mitchell's reagent and HA is at least in part due to the anthocyanin. The tannin is probably mainly of hamamelitannin type. Contain much tannin, flavonol and cyanin and but little gallic acid.
Section 2. Typical Ellagitannin Bodies, with very little or no gallotannin.					
Kousso (Pistillate inflorescences of *Brayera anthelmintica*).	Yellow either to alcoholic or aq. ext.	Yellow, brown to brownish red.	Only a poor greenish-yellow given.	Very poor display of colours.	Readily distinguished from all similar bodies known to the writer, by giving a green instead of a blue colour to ferric salts, the anthoxanthin being probably much in excess of the little ellagitannin present.

TABLE VI.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES IV AND IV*A*.—(*Continued*)

No. of section and name of substance	Colour of film given by the dil. H_2SO_4 evap. test	Reaction with lime water	Extractive tested on tile with $NaNO_2$ and 1 drop of 10% NH_3 aq.	As last but with excess of NH_3 aq. run in	Special tests, characters or remarks
Myrobalans (Fruits of *Terminalia chebula*).	Nil distinctive in aqueous extract.	Brilliant yellow, then green and finally brown. The chebulinic acid present prevents appearance of red ppt. due to ellagitannin.	Marked green (see col. VI for almost specific test).	Evanescent pink with orange in front.	Warm, moderately strong, extract, poured on to $NaNO_2$ on tile, gives a pink to crimson-red, changed to vivid green by careful addition of NH_3 aq.
Eucalyptus globulus leaves....	....	....	....	....	Perhaps contains a little gallotannin and so included in section 4.
Pomegranate (*Punica granatum*) fruit rind and root-bark.	Poor purplish or reddish.	Yellow orange and finally decidedly red.	No green given.	Usual, but not very marked pink and yellow.	If freed by the usual Pb, etc., method from tannins, etc., alkaloidal reactions can be obtained.
Algarobilla (pod of *Cæsalpinia brevifolia*).	Nil distinctive.	Evanescent orange followed by brown.	Little or nothing distinct.	Very poor colour display.	The reactions of a non-tannin phenol mask some of the ellagitannin reactions, especially the lime water ppt., but algarobilla behaves like Myrobalans with $NaNO_2$ (*alone*) q. v. Does not give green on addition of NH_3.
Section 3. Contain Ellagitannin but Gallotannin predominates					
Aleppo and Basra Galls......	Nil distinctive.	Yellow green and blue in succession.	Good green colour given.	Very rich rose-pink green and yellow.	Contain much gallic acid.
Section 4. Contain Gallotannin but Ellagitannin predominates.					
Knoppern galls.............	Nil distinctive.	Yellow, pink brown and finally a scarlet-red.	No green colour given.	Rich rose-pink, and yellow.	Sometimes gives little indication of gallotannin to the test with Mitchell's reagent and HA. but gives a very positive reaction to the ferric citrate and NaH_2PO_4 test.

Valonia (cupule or *Quercus ægilops*).	Very little distinctive.	Yellow brown and then scarlet-red.	No green given.	Rich rose-pink yellow and some green.	Readily distinguished by the rich plum-purple given by Na_2SO_3 and ext. on tile with a trace of NH_3 aq.
Eucalyptus globulus leaves....	Distinctive deep-purple to alcohol ext. Poor to aq. ext.	Yellow brown and finally characteristic red.	Poor or green colour given.	Poor colour display.	Characteristic odour. Little or no anthoxanthin or aromadendrin. Na_2SO_3 alone gives a pink. The colour-reaction to the H_2SO_4 test is due neither to the oil nor to the tannin but probably to a resin.
Divi-Divi. (pod of *C.** *coriaria*).	Aq. extract gives a beautiful purplish- or crimson-violet.	Yellow green and purple-red.	No green. $NaNO_2$ alone gives a slight pink.	Greenish-yellow and deep pink.	Trace of aromadendrin present?
Section 5. Intermediate between Sections 3 and 4, in Character.					
Kino from *Eucalyptus microcorys*.	Nil distinctive.	Yellow, green and then red.	Green colour given.	Deep green and purplish-pink both very evident.	Na_2SO_3 alone, yellowish-red. Contains no aromadendrin.
Kino from *E. maculata*	Alcoholic extract gives a yellow.	Yellow, green, brown and then red again becoming rather brownish-red.	Do.	Do.	Na_2SO_3 alone, reddish. Gives a first-rate response to the aromadendrin test (Test VI).
Section 6. Contain Tannins of Hamamelitannin Type.					
Hamamelis virginica leaves...	Good yellow film, especially in alcoholic ext.	Yellow becoming light-brown.	Green on standing.	Yellow and pink.	Characteristic odour warmed with a drop of Br. water and a little alkali. Ppt. given on boiling with dilute acid, washed and dissolved in alcohol is iron-greening.
Hamamelis bark...... ..	Gives no yellow but a poor purple.	Yellow rapidly becoming dark-brown.	No green given.	Do.	Odour given as with leaves. Does not give the iron-greening body.
Cloves (flower buds of *Eugenia caryophyllata*).	Good yellow given.	Yellow darkening rapidly.	Green slowly on standing.	Pink and deep-yellow.	Characteristic odour. Contains less tannin than commonly stated, but also contains some gallic acid and much anthoxanthin.

* Cæsalpinia.

TABLE VI.—FOR THE RECOGNITION OF THE INDIVIDUAL SUBSTANCES IN THE FINAL SECTIONS OF TABLES IV AND IV*A*.—(*Continued*)

No. of section and name of substance	Colour of film given by the dil. H_2SO_4 evap. test	Reaction with lime water	Extractive tested on tile with $NaNO_2$ and 1 drop of 10% NH_3 aq.	As last but with excess of NH_3 aq. run in	Special tests, characters or remarks
Bearberry (*Arctostaphyllos uva-ursi*) leaves.	Yellow in alcohol. extract.	Yellow, green and lastly green.	Green given.	Pink, yellow and green.	Contains quercetin and arbutin.
Logwood. (Heart-wood of *Hæmatoxylon Campeachianum*).	Rose-purple followed by yellow.	Blue-violet ppt. and often a purplish-violet filtrate.	$NaNO_2$ alone purple. Na_2SO_3 an even richer colour.	. . . .	Distinguished from most plant-substances by the purple colour given when alum is added to the ext. and from anthocyanin bodies by the negative reaction with Mitchell's reagent and HA.
Section 7. Contain Tannin of Dubious Class.					
Kino from *Eucalyptus exima*.	Alcohol. extract. gives a decided but transient yellow.	Yellow turning bright-brown.	No green with a trace of NH_3 aq.	Greenish-yellow and pink.	Gives a first-rate response to the aromadendrin test.
Kino from *E. hæmastoma*. . . .	Fairly good yellow in alcoholic extractive.	Yellow, green, red and then brown.	No green given.	Green and pink shown.	Gives a good response to the aromadendrin test. It differs from most if not all other depside gallotannin substances in giving little or no insol. ppt. to the test with $CuSO_4$ and excess of ammonia, but the sample examined contained only a little water-soluble tannin.

Stiasny's Solubility Test.—Twenty-five c.c. of a tannin solution are shaken out with 25 c.c. of ethyl acetate until the latter remains colourless. The dry residue from the aqueous layer is compared with the total soluble matter from a corresponding volume of the original aqueous solution. Owng to the fact that ethyl acetate is partly soluble in water, it is advisable to pass a current of air for about 10 minutes through the extracted aqueous solution, before taking, say, 20 c.c. for evaporation. *The difference stated in percentages gives the solubility in ethyl acetate,* which differs for the different tannins. It has been suggested (1) to replace ethyl acetate by amyl acetate, and (2) to use a specially constructed apparatus for the extraction of tannin solutions, but reference must be made to the original paper *Collegium,* 1911, 107.

Bennet's Test.—Two to three c.c. of the tannin solution are mixed with 1 or 2 drops of a 10% solution of sodium bisulphite and an equal amount of potassium chromate. All catechol tannins are said to give a greenish colour. On the other hand, some pyrogallol tannins (myrobalans, sumach, and gallotannin), give a blood-red coloration which rapidly fades to brown. Other pyrogallol tannins (valonia, chestnut, and oakwood extracts) give a deep violet which is fairly stable. The test is claimed to be useful for single tannins, or for subdividing the pyrogallol tannins into two sub-groups. It is, however, of little value in the frequently occurring case where chestnut or oakwood is adulterated with myrobalans.

Bennet's Test for Valonia, Oakwood and Chestnut Wood.—These three tannins may be distinguished from each other by the action of bromine water on a solution of the products obtained when the solid extracts are submitted to dry distillation. The extract is heated in a test-tube provided with a delivery tube, and the distillate is received in another tube. The distillate is then shaken with 15 c.c. of water, the mixture is filtered, and the filtrate treated with an excess of bromine water. Under this treatment, valonia yields a dense, yellow, crystalline precipitate of tribromophenol bromide, m. p. 139–141° (decomp.); the precipitate should be washed with cold alcohol before the melting point is determined. The distillate from chestnut tannin does not give a precipitate with bromine, whilst that from oakwood tannin yields a turbidity only.

Konstein's Test.—The tannin is precipitated by *alcutin,* (an albuminose obtained from Dr. Meyersburg, Sumper, Gasse 37,

Vienna), the solution filtered and a little strong ammonia added to the filtrate, which is then boiled and the change of colour observed.

Hoppenstedt Test.—This is said to distinguish mangrove tannin from other extracts, but can only be utilised when the mangrove extract is pure. To 25 c.c. of tannin solution (analytical strength) 25 c.c. of quinine hydrochloride (1%) solution are added, and the precipitate filtered off; 1 c.c. of glacial acetic acid is added to 5 c.c. of the filtrate in a test-tube and then 5 c.c. of ethyl acetate. The liquid is mixed thoroughly by shaking and allowed to separate into layers. With mangrove the lower layer is coloured a strong yellow brown; with the other tannins the layer is colourless.

Dieterich's Test.—This serves to detect the presence of gambier. Add 5 c.c. of alcohol to 5 c.c. of the tannin solution. After shaking add 1 c.c. of 1% potassium hydroxide solution. Then add 10 c.c. of petroleum spirit, mix, and allow the layers to separate. With gambier the upper layer exhibits a strong green fluorescence. It is claimed that this test will apply to mixtures (Hoppenstedt.)

Hoppenstedt's Test for Hemlock.—To 50 c.c. of the tannin solution add 10 grm. of calcium chloride (anhydrous), shake until dissolved, cool, and filter. Place 5 c.c. of the filtrate in a test-tube, and add 1 c.c. of glacial acetic acid and then 5 c.c. of amyl acetate; shake and allow the layers to separate. With hemlock the upper layer is coloured a strong yellow brown; with other tannins it is colourless. This is claimed to be efficient in identifying hemlock in mixtures. (*J. Amer. Leather Chem. Assoc.*, 1912, **7**, 172).

Eitner and Philip's Sulphide Test.—Add 2–3 drops of strong sulphuric acid to 25 c.c. of a strong tannin solution (2.5%) in a flask and boil for 1–2 minutes, cool, add about 5 grm. of salt, shake, allow the mixture to stand for 5–10 minutes, and filter off the precipitate. In a test-tube add 10–15 drops of ammonium sulphide solution to about 15 c.c. of water and then 2–3 c.c. of the above filtrate. All pyrogallol tannins give a copious precipitate of varying colour, whilst most catechol tannins give no precipitate even after standing over night. Procter (*ibid.*) states that mimosa and malet behave like pyrogallol tans in this test, and that they can therefore be easily detected, even in mixtures with other catechol tans. It must be remembered, however, that other tannins (pine, catechu, and gambier) are also precipitated by ammonium sulphide solution. (*J. Amer. Leather Chem. Assoc.*, 1909, **4**, 249.)

Assuming that the total tannin as returned by the official method really consists of tannin and certain neutral substances, R. Vanicek (*Zeitsch. angew. Chem.*, 1913, **26**, 68) proposes to estimate the acid in the original solution by titrating with N/10 sodium hydroxide, using phenolphthalein by the "spotting out" method. A portion of the original solution is then detannated with gelatin, and the titration repeated. The difference is calculated as tannin, a predetermined factor being used for each different tannin under examination. The values of the available tests stand in the following order:

Solubility test
Formaldehyde test
Acetic acid and lead acetate test
Aniline test
Bromine test

These tests must be taken in conjunction with the amount of tans and non-tans and their ratio, and also the Löwenthal figure. The advent of sulphite-cellulose extracts, and the fact that their active constituent is returned by the official method as tannin, is one of the chief reasons for the Löwenthal method.

In extreme cases the refractometer method may be of some value, and further work has been done in this direction by Kubelka. (*Collegium*, 1914, 151.)

As has been before pointed out, when the tannin material is to be used for other purposes than that of tanning skins, the problem presented to the analyst is of a different order, and this must always be remembered when deciding upon the methods to be used to estimate the tannin present. Thus in dyeing silk, the weight-giving properties of the extract in combination with its colour alone and in the presence of mordants, are of special significance.

It has been shown by Knowles (*J. Soc. Dyers and Col.*, 1912, **28**, 174), that the results obtained by the official method (A. L. T. C.) do not correspond with the results obtained on cotton with subsequent saddening with ferric sulphate, nor does the percentage of iron taken up agree with the tannin present, as estimated by the official method. Again, in the dyeing of silk, some tannin-extracts although showing a high percentage of tannin, are quite unsuitable for certain purposes.

The chief tests for the differentiation of tannins have been conveniently collected by Stiasny in the following tables (*J. Amer. Leather. Chem., Assoc.*, 1914, **9**, 22). An interesting use is made of the formaldehyde test at both 15 and 20 minutes.

Group 1.—Complete precipitate; the filtrate gives neither gelatin nor iron reaction.

Tests for confirmation; bromine test (precipitate) and acetic acid and lead acetate test (no precipitate).

Group 2.—No precipitation during 15 minutes' boiling.

Test for confirmation: bromine test (no precipitate); ammonium sulphide test (precipitate).

Group 3.—Considerable precipitate during boiling, but distinct iron reaction of the filtrate.

To Group 1 belong: quebracho, mangrove, ulmo, gambier, pine-bark, hemlock, mimosa, malet.

To Group 2 belong: oak-wood, chestnut-wood, valonia, myrobalans.

To Group 3 belong: oakbark, *Pistacia lentiscus*, sumach, divi-divi, algarobilla, teri, bablah, galls.

It having been found to which group the tannin belongs, the following tests are made in each group:

Further Testing of Group 1.—The ammonium sulphide test allows a sub-division, in so far as no precipitate is obtained with quebracho, mangrove, ulmo, gambier, pinebark, hemlock (Group 1a), whilst a precipitate is shown by mimosa and malet (Group 1b).

Group 1a is also characterised by the green coloration produced with iron alum.

Group 1b gives a bluish violet with iron alum.

The further way of identifying the tannin in 1a or 1b, demands the carrying out of all the tests mentioned in previous papers and summarised in Table II. This table also contains the gallic acid value of 1 grm. of the tannin and the proportion of tans to non-tans in the tanning material. The Mo-figures found by Lauffmann are also given in the table.

Further Testing of Group 2.—The acetic acid and lead acetate test allows a subdivision, as no coloration is given on adding iron alum to the filtrate of the lead precipitation, in the case of oak-wood and valonia (Group 2a); whilst a more or less distinct violet coloration is obtained with myrobalans and chestnut (Group 2b).

TABLE I

Fifty c.c. tannin solution (0.4%) boiled with 25 c.c. of the formaldehyde—HCl mixture for 30 minutes, thoroughly cooled and filtered.

Complete precipitate: Filtrate + iron alum + sodium acetate: no violet coloration.		Noprecipitate after 15 minutes boiling.		Considerable precipitate after 15 minutes boiling; deep violet coloration of the filtrate + iron alum + sodium acetate.	
GROUP I		GROUP II		GROUP III	
Confirming tests: + bromine: precipitate. + acetic acid + lead acetate: no precipitate. 25 c.c. tannin solution (2.5 %) + a m m o n i u m-sulphide test.		Confirming tests: + bromine: no precipitate. Ammonium-sulphide test: precipitate. 5 c.c. tannin solution (0.4 %) + acetic a c i d + lead-acetate test. The filtrate of the precipitate gives + iron alum.		5 c.c. tannin solution. (0.4 %) + bromine test.	
No precipitate.	Precipitate.	No coloration.	Violet coloration.	Precipitate.	No precipitate.
GROUP Ia	GROUP Ib	GROUP IIa	GROUP IIb	GROUP IIIa	GROUP IIIb
Confirming tests· + iron alum.		Oakwood Valonia	Chestnut Myrobalans	Oakbark Pistacia	Sumach Divi-divi Algarobilla Galls Bablah Teri
green Quebracho Mangrove Ulmo Gambier Pine-bark Hemlock	bluish violet Mimosa Malet				

The sugar content of tannin extracts plays an important part in actual tanning. Apart from this, the variations observed in the amounts present may also indicate the nature of the extract, and therefore be useful to the analyst.

The following table due to Paessler (*Collegium*, 1913, 187) indicates the differences observed when using pure extracts; the "shake" method was adopted for the separation of the tannin.

Material	Non-tans	Monosaccharides	Disaccharides	Total sugars
Pine bark	93	33	14	47
Oak bark	74	30	0	30
Sumach	74	17	2	19
Myrobalans	49	18	0	18
Divi-divi	55	11	4	15
Mimosa	39	6	85	14.5
Valonia	49	12	0	12
Trillo	44	7.5	1.5	9
Mangrove	30	2	1	3
Quebracho wood	14	1	1	2

TABLE II

	Formaldehyde test		Bromine test	Ammonium-sulphide test	Lead-acetate test. Filtrate + NaOH	Acetic acid + lead acetate test		Ethyl-acetate figure	Alcohol figure	Gallic acid value of 1 gm. tannin. (See *Collegium*, 1909, 191)	Tans / Non-tans	Molybdenum figure (Lauffman)
	During 15 min. boiling	Filtrate + iron alum + sodium acetate					Filtrate + iron alum					
Quebracho	pp.	No coloration	pp.	No pp.	Yellowish	No pp.	Green	70–80	0–5	0.59	8.0–10.0	25–40
Sulphited-quebracho.	pp.	No coloration	pp.	No pp.	Yellowish	No pp. (but $PbSO_4$)	Green	0–70	0–5	0.59	Depends on the method of sulphiting	0–20
Mangrove	pp.	No coloration	pp.	No pp.	Colourless	No pp.	Green	0–5	0–5	0.68	2.5–4.0	100–135
Ulmo	pp.	No coloration	pp.	No pp.	Yellowish	No pp.	Green	70–80	0–5	....	8.0–10.0	42
Gambier	pp.	No coloration	pp.	No pp.	....	No pp.	Green	50–65	5–10	0.56	1.2–1.5	0–13
Mimosa	pp.	No coloration	pp.	pp.	Colourless	No pp.	Deep bluish violet	30–40	0–5	0.53	2.0–3.0	110–130
Oakbark	pp.	Violet	pp.	pp.	Colourless	pp.	....	12	17	....	1.0–1.5	135
Hemlock	pp.	No coloration	pp.	pp. (after standing over night)	Yellowish	No pp.	Green	18	9	....	1.0–2.0	65–85
Pistacia	pp.	Deep bluish violet	pp.	pp.	Yellow	pp.	Green & violet	3	29	....	....	....
Chestnut	No pp.	Deep bluish violet	No pp.	pp.	Colourless	pp.	Very faint violet	0–16	10–20	0.56–0.66	2.0–3.5	180–225
Oakwood	No pp.	Deep bluish violet	No pp.	pp.	Colourless	pp.	Colourless	0–12	20–30	0.5–0.56	1.0–2.0	175–210
Myrobalans	pp.	Deep bluish violet	No pp.	pp.	Colourless	pp.	Violet	30–50	0–15	0.55–0.60	1.5–2.5	80–140
Sumach	pp.	Deep bluish violet	No pp.	pp.	Yellow	pp.	Violet	40–60	5–20	0.65–0.69	1.5–1.8	125–155
Valonia	Turbid	Deep bluish violet	No pp.	pp.	Colourless	pp.	Colourless	5–15	20–40	0.55–0.63	2.0–3.0	222
Divi-divi	Turbid	Deep bluish violet	No pp.	pp.	Colourless	pp.	Violet	30–50	0–10	....	2	....
Algarobilla	Turbid	Deep bluish violet	No pp.	pp.	Colourless	pp.	Violet	50–60	0–5	....	2	....
Wood pulp	No pp.	No colouration	No pp.	Not characteristic	Deep yellow	No pp.	Colourless	0–5	30–70	0.09–0.14	0.75	....

Brissemoret's Test.—The tannin is dissolved in sufficient alcohol or ethyl acetate, and to this is added glacial acetic acid containing a small quantity of ferrous sulphate. On pouring sulphuric acid containing a little ferric sulphate into this mixture so as to form a separate layer, the characteristic colours are produced at the plane of contact of the two layers:

Ellagic acid	yellowish-green
Ellagitannin	yellowish-green
Gallotannin	yellow
Caffeetannin	wine-red
Aniatannin	wine-red
Quebrachotannin	wine-red
Tannin of male fern	scarlet
Tormentilla tannin	violet-red
Rhatany roots	bordeaux-red
Cutch	bordeaux-red
Kola	bordeaux-red
Guarana	bordeaux-red

For further details reference should be made to the original paper: (*Bull. Soc. Chim.*, 1907, (IV), **1**, 474).

Grasser's Test.—Hydriodic acid is used as a reagent (*Collegium*, 1911, 46) with the following results:

Pine	violet
Algarobilla	green
Chestnut extract	dark green
Maletto	dark green
Myrobalans	dark green
Quebracho extract	dark green
Sumach	dark green
Sumach extract	dark green
Divi-divi	no coloration
Oak	no coloration

Schneider-Seiwert's Test.—Mordanted cotton-wool gives the following results:

Aluminium.

(*a*) Yellowish-green: Myrobalans, Divi-divi, Algarobilla, Sumach, Knoppern, Valonia.

(*b*) dark yellow-brown: Chestnut-wood, Oak-wood, Chinese galls, Maletto-bark.

(*c*) greyish-brown: Bablah, Willow-bark, Oak-bark.

(*d*) reddish-brown: Kino, Hemlock-extract, Mimosa, Pine-bark, Quebracho, Mangrove.

(*e*) light-brown: Gambier.

(*f*) yellow-brown: Cutch.

(*g*) orange-brown: Ratania-root.

Titanium.

(*a*) light yellow-brown: Chestnut-wood, Algarobilla, Bablah, Myrobalans, Chinese galls.

(*b*) brown-orange: Oak-wood, Oak-bark, Sumach, Divi-divi, Valonia, Knoppern.

(*c*) light yellow-brown: Hemlock-extract, Mimosa, Maletto.

(*d*) red-brown: Gambier, Quebracho, Mangrove.

(*e*) dark red-brown: Cutch.

For further details reference must be made to the original: (*Collegium*, 1911, 282).

Nierenstein's Test.—Diazobenzene chloride (½% solution) precipitates only catechol-tannins.

Eitner, Meerkatz and Philip's Test.—Ammonium sulphide gives the following precipitates:

Chestnut-wood	brown, turns red.
Oak-wood	yellow, turns bordeaux red.
Oak-bark	yellow, turns brown.
Valonia	yellow, turns light brown.
Knoppern	yellow, turns red-brown.
Myrobalans	greenish-yellow, does not change.
Divi-divi	light yellowish-green, does not change.
Hemlock	only on standing yellow-brown.
Maletto	yellowish-brown, does not change.
Quebracho	no precipitate.
Mangrove	no precipitate.
Gambier	no precipitate.
Cutch	no precipitate.

Pollak's Test.—The addition of ammonia to the aqueous extract gives:

with Quebracho: white precipitate.

with Mangrove: pink-coloured precipitate.

Hoppenstedt's Test.—This test describes a series of detailed experiments for the identification of mangrove, gambier, hemlock and chestnut-oak, and reference should be made to the original: (*J. Amer. Leather Chem. Assoc.*, 1912, **7**, 97 and 143).

QUANTITATIVE ESTIMATION OF TANNINS

A number of methods have been described for the quantitative estimation of tannins. Dekker (*Chemie. der Gerbstoffe*, 481–550) accounts for 86 methods, but, from a historical study made by the

writer, it appears that as many as 94 methods have actually been elaborated.

The underlying principles of these methods are:

1. Oxidimetric methods.
2. Absorption of the tannins by hide-powder.
3. Absorption of the tannins by other organic substances.
4. Precipitation of the tannins by organic salts.
5. Precipitation of the tannins by inorganic salts.
6. Iodometric methods.
7. Colorimetric methods.
8. Absorption of the tannins by inorganic substances.
9. Polariscopic methods.
10. Precipitation of the tannins by electricity.
11. Refractometric methods.
12. Specific gravity methods.

The following points must be carefully considered when deciding on the selection of any process of analysis for special purposes. It must be remembered that the chemistry of tanning is so involved that only practical trial and experience can confirm the relative values of different tannin materials. For the purpose of the tanner a purely empirical method of analysis is still adopted. The "active" tannins are absorbed under certain artificial, but definitely laid-down conditions. This has been found to be absolutely necessary in order that different analysts may obtain similar results when reporting on the value of these materials. Under these specific conditions it is assumed that the value of any extract or tanning material may be ascertained and results obtained which will indicate its relative value in terms of this absorption. The necessary details are confirmed and varied by Conferences, both in Europe and America.

For the dyer these "official" figures may have little value in cases in which dyeing black on iron mordants, or the weighting of the silk libre is the object aimed at, or when mordanting cotton for dyeing fight shades with basic dyes.

Under these conditions a process which is in some way comparable to the conditions in practice may be more suitable as in the corresponding case of the leather industry. The hide-powder results may therefore be of minor value to the "lake manufacture," the black silk dyer or the ink-manufacturer and for special purposes one of the other

processes of analysis may be selected and supplemented by some practical test, such as the actual weighting of a silk yarn under standard conditions, or the dyeing of the same on one or more mordants, or the treating of cotton yarn in some similar way to that adopted in working on the large scale.

Owing to the highly organised condition and official status of the hide-powder process these points are sometimes overlooked.

Sampling of tannin materials is often a troublesome operation, and, together with the difficulty attending complete extraction, is a fertile source of error. The official associations have stringent conditions which cover this operation and these must be referred to.

When available, a steel mill is the best means of roughly pulverising most tannin materials. With the exception of barks, the grinding can be effected by a disintegrator with fine screens, great care being taken to prevent the escape of dust. Barks may be sampled by cutting each fragment with a small circular saw or rasp driven by a lathe, and collecting the dust.

In sampling valonia care must be taken to get a due proportion of the beard, and in taking myrobalans it must be remembered that the bad berries are light and apt to work towards the top of the bag. The sample being partly reduced by one of the above means, the moisture (usually 15 to 16%) must be estimated by drying at 100°.

Sampling Extracts in Barrels.—Lepetit (*Collegium*, 1910, 382) proposes that in the place of the official regulations the total number of casks to be sampled shall be the square root of the total number less one, instead of 5%. The barrels are rolled a distance of 20 metres and left to stand for a few minutes first on one end and then on the other. The bung is removed and 2 buckets full of extract removed, the contents of the cask being shaken before removing the sample to be tested. This method is said to occupy much less time and to be more efficient than the official European method.

The extraction of tannin matters is better effected by treating the sample at once with a large quantity of water than by repeated treatment with smaller quantities. Reference may be made to the official methods of extraction for analysis.

The analysis of chestnut wood is said (Alsop. *J. Amer. Leather Chem. Assoc.*, 1909, **4**, 95) to give difficulty owing to substances which yield almost indefinitely to the extraction.

Tannin solutions free from colouring matters have been prepared by adding zinc sulphate and ammonium sulphate to the solution; tannate of zinc is thus precipitated, which is washed with a very dilute solution of ammonia. It is then suspended in 5 times its volume of water and decomposed by dilute sulphuric acid. Barium sulphide is added till no further precipitate is formed. The precipitate, which consists of zinc sulphide and barium sulphate, is removed, and an almost colourless solution of tannin remains. This method has been applied to the manufacture of *colourless extracts*.

Tan liquors have been decolorised by treating with lead nitrate, and afterward adding alum and borax. Strontium hydroxide and carbonate have also been used for the precipitation of the colouring matters, sulphuric acid being added to the filtrate to remove the excess of strontium salt. Clarification is also accomplished by electrolysing the liquid to which has been added oxalic acid and sodium chloride. Sumach may be decolorised with fair results by the use of ox blood.

Grasser recommends a special apparatus for tannin extraction (*Collegium*, 1910, 345) in place of Procter's sand-filter apparatus. It consists of 2 copper cylinders, the inner one being supported by three projections. The lower extremity of the inner tube is packed with cotton wool. The material to be extracted is contained in this inner tube. Water, at a gradually increasing temperature (from 25°–60°) is poured on the substance until 400 c.c. have passed, when the litre flask at the bottom is replaced by an Erlenmeyer flask containing 250 c.c. of water. A condenser is attached and extraction takes place for one hour and the extract is added to the first 400 c.c. Re-extraction takes place with a fresh 250 c.c. of water until the material is exhausted. With spent material it is sufficient to boil up with 300 c.c. of water.

Of the numerous methods which have been devised for the assay of tannin matters, many have been based on the principle of precipitation of the tannin by a solution of gelatin or its absorption by a gelatinous substance. In some cases the weight of the precipitate formed, or the increase in the weight of the solid gelatinous substances has been found, but the better plan is to ascertain the quantity of tannin precipitated by comparing the solution after the treatment with the original untreated solution. This is done by

Hammer by taking the sp. gr.; by Simand and Weiss by weighing the solid matter left on evaporation; and by Löwenthal by determining the volume of standard permanganate solution decolorised by the solution before and after removal of the tannin. These methods, which appear simple enough in principle, are in practice surrounded with very considerable difficulties, especially when gallic acid or other impurities are present. For instance, the disturbing action of gallic acid on the ordinary hide-power process may be indicated by the following figures, the estimation of pure gallic acid being attempted in different ways with the following results:

Dreaper's method (lead separation) shows........	0.0% tannin.
Dreaper's method (hide powder separation)......	45.6% tannin.
Hide-powder process..........................	27.0% tannin.

These variations were found, on further investigation, to be due to two distinct causes: 1. Absorption of gallic acid by hide powder; 2. solubility of hide powder in gallic acid solution. In the first case neither of these defects is present; in the second the absorption of the gallic acid is estimated; in the third this result is modified (to the extent of 18.6%) by the solubility of the hide powder which partially "corrects" the error in the second case, as the non-tannins are estimated by direct weighing (Dreaper, *Chem. News*, 1904, **90**, 3).

These results are given, as they indicate the nature of the controlled action of the recently-introduced chrome or "treated" hide powders. The chroming action, by rendering the hide powder less soluble, tends to correct the above error, (18.6%), and, at the same time, by reducing the hydration of the hide material, correspondingly reduces the ratio of the absorption of gallic acid without materially decreasing that of the tannin. The result of variations in the hydrogel state of such precipitating media (as gelatin or albumin) has been investigated by Dreaper and Wilson (*J. Soc. Chem. Ind.*, 1906, **25**, 515), and reference to the results obtained will give the analyst some idea as to the nature of these reactions, and the absolute need for some artificial control over the absorption by hide powders under varying conditions. The figures given above represent the extreme errors possible with this process, for it was found that in the presence of tannin in the solution the hide powder was apparently not so readily hydrated. The portion dissolved from the hide powder and the absorption of gallic acid were both reduced. A modified hide powder must be looked for as a standard, which will

give equal results to the above separation method when using gallic acid alone. It may then be assumed that in mixtures of tannin and gallic acid no absorption of the latter, or solution of the hide-powder substance will take place. Owing to this varying action a blank experiment with distilled water may be quite useless for estimating the soluble matter in any sample of hide powder under the conditions of analysis and such a test should be discarded.

Only certain portions of the hide can be used in the preparation of hide powder, and all samples used are prepared under standard conditions and in bulk. From the foregoing considerations the empirical nature of this method of analysis is obvious.

The absorption of colouring or other substances by hide powder or gelatin may introduce a serious error, as they may be weighed as tannin. The fact that these substances are absorbed under certain conditions by these precipitating media is one of real concern to the analyst, and must be considered when deciding on any method of analysis. Where this disturbing influence is present a process should be selected which does not depend on the direct weighing of the separated tannin. In the case of the control of solutions, or vats in industrial operations this may be necessary. It may be advisable in such cases to assay the original tannin material by the hide-powder process, and also by the one selected for use in the dye house or tanning yard, so that the latter method may be standardised in terms of the hide-powder processes. The mere "carrying down" of other materials, coloured or otherwise of an inactive nature, by, say, a copper, or antimony lake need not necessarily interfere with the assay of the tannin where the resulting precipitate is not weighed.

The influence of third substances (such as acids or salts) on the proportion of gallic acid carried down, or absorbed by, the gelatin coagulum or hide powder may be both marked and definite in its nature.

In fact, gelatin nearly free from ash will not precipitate tannin (Weiske, *Zeitsch. Phys. Chem.*, 1891, **7**, 460) which shows that slight variations in the conditions, such as the varying presence of salts, may materially alter the composition of the precipitated coagulum, and emphasises the absolute need for an official or recognised method of analysis based upon conditions which will reduce these errors to a minimum, or at any rate standardise them (see J. T. Wood, *Collegium*, 1908, 494; also *J. Soc. Chem. Ind.*, 1908, **27**, 384).

THE OXIDATION METHOD OF TANNIN ASSAY

This process, which was first worked out by Löwenthal, is based on the fact that tannin is oxidised in acid solutions by permanganate, though the slowness of the oxidation and the want of definition of the end-point render the method unsuitable without modification. By addition of a considerable quantity of indigo the oxidation of the tannin is controlled, and the end-point is rendered more definite. As solutions of commercial tannin matters contain oxidisable matters other than tannins, it is necessary to separate these and titrate a second time, in order to ascertain the volume of permanganate actually required by the tannin present. This separation may be effected by digestion with hide powder, or by a solution of gelatin, the use of which was first suggested by Estcourt.

The compounds of gelatin and tannin have been studied by J. T. Wood (*Collegium*, 1908, 257 and 269), and reference to the results will show that the ratio of tannin to gelatin is not a constant, the tannin being precipitated in greater ratio in stronger solution. Washing also influences the ratio, it being in one case reduced from 310 to 212. The composition of the gelatin coagulum formed has been studied by Trunkel (*Biochem. Zeit.*, 1910, **26**, 458). It varies greatly according to the conditions of coagulation.

In practice, Löwenthal employs a mixed solution of gelatin and common salt, to which a small quantity of sulphuric or hydrochloric acid has been added. In using this form of process it is generally necessary to let the mixture stand several hours in order to obtain a clear filtrate, besides which the gelatin substance remaining in solution has a slight, though generally negligible reducing action on the permanganate. In some cases, even after long standing, filtration is very tedious, and it has also been proved by F. Simand (*J. Chem. Soc.*, 1883, **43**, 1237) that a certain proportion of the tannin and gelatin precipitate, varying with the acid and kind of tannin present remains in solution, and hence that the results obtained by the process are below the truth.

On account of these objections to Löwenthal's process, Procter proposed a modification in which the excess of gelatin is removed by saturating the liquid with common salt, and the filtration is facilitated by the addition of kaolin. A perfectly clear filtrate wholly free from tannin, and nearly so from gelatin, is thus obtained without difficulty. The following were the details given:

Ten grm. of sumach or valonia or 20 grm. of finely ground bark are exhausted with water.

(*a*) Five c.c. of the solution for analysis are run into a porcelain basin and diluted to 750 c.c. by addition of distilled water and 20 c.c. added of an indigo solution, a litre of which contains 5 grm. of the purest indigo-carmine, and 50 c.c. of concentrated sulphuric acid. The indigo-carmine (sodium sulphindigotate) must be of such quality that the solution when oxidised by permanganate is a pure yellow colour, free from a trace of brown or orange. Indigo-purple, which gives brown oxidation products, interferes with the accuracy of the analysis. The indigo solution should be of such strength that 20 c.c. diluted to 750 c.c. with water, shall require from 14 to 16 c.c. of standard permanganate for its oxidation. A solution containing 1 grm. of potassium permanganate per litre is then run in very slowly, drop by drop with vigorous stirring, until the liquid becomes transparent, when the addition is continued more cautiously, with occasional pauses, until the clear yellow liquid appears of a faint pink colour on the margin. The titration is repeated, the volumes of permanganate required in the two cases being added together and called *a*.

In employing the oxidation process, the volume of permanganate required by the tannin should *in no case* exceed 2/3 of that reduced by the indigo. If the result of the titration shows that this proportion has been exceeded, the experiments must be repeated with a smaller quantity of the tannin solution.

(*b*) For the gelatin separation 50 c.c. of the tannin solution should be mixed in a flask with 28.6 c.c. of a freshly-made and filtered solution of gelatin. The gelatin solution is prepared as follows: 2 grm. weight of good gelatin is allowed to swell in distilled water for a few hours, then melted by immersing the flask in boiling water, and the resultant solution made up to 100 c.c. After shaking, the liquid is saturated with common salt, which increases the volume to 90 c.c. 10 c.c. of dilute sulphuric acid (containing 1 volume of the concentrated acid in 10) should next be added, and then about 10 grm. of pure kaolin or barium sulphate. In this connection reference must be made to B. Hunt (*J. Soc. Chem. Ind.*, 1885, **4**, 263) who has indicated that the excessive quantity of salt recommended by Procter causes the precipitation of a notable quantity of gallic acid when much is present. Hence he prefers to mix 50 c.c. of the tannin

solution with 25 c.c. of a 2% solution of gelatin, and then add 25 c.c. of a saturated solution of common salt containing 50 c.c. of strong sulphuric acid per litre. Kaolin is next added, and the mixture well shaken and filtered and Procter's method of operating is adhered to in all other respects. Hunt's modification is approximately a return to Löwenthal's original method, and introduces its attendant error. In the presence of gallic acid the gelatin separation is a doubtful one in all cases. The flask should be vigorously shaken for a few minutes, and the liquid passed through a dry filter. This is effected rapidly, and the filtrate is perfectly clear. Two quantities of the filtrate of 10 c.c. each (= 5 c.c. of original infusion) are then treated with indigo solution, and titrated with standard permanganate as before, the result being called *b*. The difference $(a-b)$ between the volume of permanganate employed for the 2 quantities of unprecipitated tannin infusion (*a*), and that decolorised by the 2 portions of the filtrate, gives the volume of permanganate solution decolorised by the tannin in 10 c.c. of the original infusion.

(*c*) 10 c.c. of an N/10 solution of oxalic acid (6.3 grm. of crystallised oxalic acid, $C_2H_2O_4.2H_2O$, per litre) are diluted with distilled water to about 500 c.c. warmed to about 60°, 20 c.c. of pure dilute sulphuric acid added, and standard permanganate run in with constant stirring till a pink coloration, remaining permanent for 1 minute, shows that oxidation is complete. The volume of permanganate consumed, which is called *c*, is evidently that required for the oxidation of 63 mg. of crystallised oxalic acid.

The proportion $c : (a - b) = 63 : x$ will give a number of mg. of oxalic acid corresponding in reducing power to the tannin in 10 c.c. of the infusion assayed. If 10 grm. of the sample were extracted and the solution made up to 1 litre, 10 c.c. of the infusion represents 0.1 grm. of the tanning material, and hence the number of mg. of oxalic acid will be *the percentage of tannin expressed in terms of crystallised oxalic acid.* It is frequently convenient to express the results of the assay in this way, since what is required in practice is not the absolute weight of tannin in the various materials, but their *comparative* value in terms of tannin. It is impossible to express the results of tannin assays in actual percentage of tannin; unfortunately the different varieties of tannin have different reducing powers, and the expression of the results of the assay of oak-bark or cutch in terms of gallotannin would be misleading. The expression of assays of

all kinds of tannin matters is, therefore, made in terms of oxalic acid.

Von Schroeder has suggested the use of commercial gallotannin, the moisture in which has been estimated by drying at 95°, and which has been proved to contain not more than 5% of non-tannin matters unprecipitable by hide or gelatin as a standard; dividing the result obtained by 1.05 to allow for the slightly higher reducing power of the impure tannin. Procter has proposed the use of gallic acid for standardising the permanganate, as it is readily obtained pure, and is oxidised in the presence of indigo in a manner very similar to gallotannin.

The Yorkshire College method, as given by Procter (*Leather Industries Laboratory Book*), varies in details from the others. It is given here and recommended for general use, when it is decided to employ this method.

The solutions required are; I. Potassium permanganate 0.5 grm. per litre, freshly prepared, if possible.

II. Pure indigo carmine solution (potassium or sodium sulphindigotate) 5 grm., and concentrated H_2SO_4, 5 grm. per litre. 25 c.c. of this solution should equal 30 c.c. of the permanganate.

III. Solution of "pure" tannin; or gallic acid may be substituted on account of its purity.

The tannin solution is never quite pure, and must be standardised by the hide-powder process. It must not show less than 90–95% tannin, and a correction must be made for this.

25 c.c. of the indigo carmine solution is mixed in a beaker with about 750 c.c. water, and the permanganate added, drop by drop, till a pure yellow colour is obtained. Care has to be taken to stir the solution in a constant and regular way. The titration is then repeated in the presence of 5 c.c. of the tannin solution, or the tannin solution under examination. These figures give the total astringent present in terms of tannin. To obtain the astringent taken up by hide powder, which may be less than that estimated by the permanganate, the usual hide-powder separation may be adopted.

The gelatin separation method may be used where the proportion of gallic acid is small, Hunt's modification being selected for use.

Solutions required:

1. Pure gelatin, 2 grm. per 100 c.c.

2. Saturated solution of sodium chloride containing 50 c.c. sulphuric acid per litre.

To 50 c.c. of the tannin solution are added 15 c.c. of gelatin, 25 c.c. of the salt solution and about a teaspoonful of kaolin, and the whole is well shaken for 5 minutes and filtered. Double the original volume taken for examination is used for titration.

The actual reduction equivalents of the different kinds of tannins are imperfectly known, and the greatest caution must be observed in their use. Neubauer states that of gall tannin as 41.57; that is, 41.57 grm. of gall tannin possess the same reducing power on permanganate that is possessed by 63 grm. of crystallised oxalic acid ($C_2H_2O_4.2H_2O$), or 56 grm. of iron in the ferrous state, or that 41.57 grm. of gall tannin decolourise a volume of permanganate solution yielding 8 grm. of available oxygen. Neubauer's equivalent for gallotannin has been confirmed by Ishikawa (*Chem. News*, 1888, **42**, 274) who found 41.688 as the figure for the tannin of *Kibushi*, or Japanese gall-nuts. Councler and von Schroeder, on the other hand, find the equivalent to be only 34.25. This discrepancy has been shown by von Schroeder to be due to the different manner in which the permanganate was added in the titration. Neubauer employed the "drop method," whilst Councler and von Schroeder added the solution in successive quantities of 1 c.c. with a short interval between each addition. This modification seriously affects the volume of the standard solution required. It is clear that by expressing such results in figures showing the second or third decimal places, authors show a lack of appreciation of the probable error of the method. For oak-bark tannin, Neubauer gives the equivalent 62.36, which is confirmed by Oser's figure, 62.35, and approximately by that of Simand, 61.1. The reduction-equivalents of other varieties of tannin are uncertain. Oser's and Neubauer's figures for oak-bark tannin show a reducing power nearly identical with that of oxalic acid (= 63), and hence the results of the titration may be conveniently expressed in terms of oxalic acid. An alternative plan is to state the strength of the tannin matter in terms of "oxygen consumed." Each 1 c.c. of a solution of potassium permanganate (containing 1 grm. of the salt per litre) which may have been decolorised by the tannin, represents 0.000253 of "oxygen consumed," or 0.00199 (practically 0.002) grm. of crystallised oxalic acid. Neubauer's equivalent for gall tannin is practically two-thirds of the

bark and oxalic acid figures. The first figure is applicable to galls, and probably to divi-divi, sumach, and myrobalans; the second to oak-barks, and probably to oak-wood, valonia, chestnut extract etc. Gallic acid consumes a greater volume of permanganate than the tannin from which it is derived. Hence, as commercial tannin is often largely contaminated with gallic acid, it not infrequently shows over 100% of tannin when assayed. Mixtures of tannins with gallic acid cannot be directly estimated by the volumetric process, for 1 grm. of the dry acid reacts with the same quantity of permanganate as 1.505 grm. of dry tannin.

This method (Löwenthal) has been criticised by Procter and Hirst (*Collegium*, 1909, 193). The non-tannins in the case of gelatin precipitation are too high, though they are approached, or even exceeded in some cases, by the unchromed hide-powder (shaking) method. When chromed powders are used the results are invariably lower. Paessler's lightly chromed powders (dry) are said to be equally suitable to the official powder (chromed) for the Löwenthal method or to the Kopecky air-dried chrome leather machine shavings. In testing the tannin liquors 7 grm. of the dry powder are taken, and a little kaolin added to 100 c.c. of the liquor. This is well mixed by hand-shaking, and then by treatment for 10–15 minutes in a shaking machine. After passing through filter-paper until clear 5 c.c. are taken, 20 c.c. of indigo added, and titrated with the permanganate solution. The latter is titrated against a standard gallic acid solution, and the results expressed "in terms of gallic acid." The estimation of the non-tannins has also been the subject of a special study by H. R. Zeuthen (*Collegium*, 1908, 366).

According to Cech, no interference in the estimation of tannin by permanganate is produced by the presence of acetic acid, citric acid, tartaric acid, malic acid, cane-sugar, dextrin, gum, fat, caffeine, or carbamide, provided the solution be diluted as directed.

The permanganate, and possibly all other, processes for the assay of tannin-matters are merely comparative, and give results useful only as a means of comparing the qualities of samples of material of the same character. Thus, bark may be compared with bark, and valonia with valonia, and so on, but *all cross comparisons are impossible.* Even if the exact percentage of tannin could be calculated, the practical and commercial value of tannin materials does not depend on the quantity of tannin only, but on the colour,

weight and quality of the leather produced, though the same process should give results of approximate accuracy when applied to different materials containing the same variety of tannin.

P. Sisley (*Bull. Soc. Chim.*, (iii), 1909, **9**, 755) precipitates the tannin as a zinc salt, and oxidises the latter with permanganate. An ammoniacal solution of zinc acetate is used for the precipitation, made by dissolving 40 grm. zinc oxide in hot, dilute acetic acid (65 c.c. glacial acid and 50 c.c. water) and adding excess of ammonia. The tannin solution is treated with zinc solution, and the precipitated zinc compound rapidly filtered and washed with dilute ammonia. In this way the gallic acid and other impurities are removed. The precipitate is then washed into a porcelain basin and titrated with potassium permanganate.

The following figures given by Procter show the results to be expected when applying the permanganate process to the assay of various tannin matters.

	In terms of oxalic acid	
	Tannin, %	Other oxidisable substances, %
Valonia; good Smyrna	29.1	2.3
Valonia; good Smyrna	30.7	2.1
Valonia; good Smyrna	30.5	1.9
Valonia; good Smyrna	32.6	2.7
Hungarian larch extract	14.78	1.95
Hungarian larch extract	18.08	2.33
Chestnut-wood extract (sp. gr. 1.205)	25.53	3.68
Pegu cutch	63.59	2.45
Spent liquor	0.12	11.0

The permanganate process has been applied by A. Hill to the estimation of tannin in *tea* (*Analyst*, 1881, **6**, 95). The average proportion of tannin, in terms of oxalic acid, found in the 32 samples of tea examined was 14.8%, the extreme results being 6.18% in black Assam tea and 26.90% in a black caper tea. Other estimations of the proportion of tannin in tea have been made by O. Kellner (*Landw-Versuchs Stat.*, 1886, 370) and J. F. Geisler, (*Analyst*, 1884, **9**, 220).

The following figures, due to B. Hunt (*J. Soc. Chem. Ind.*, 1885, **4**, 264) show the insoluble matter and total extract of various com-

mercial tannin materials, and the oxalic acid equivalents of the total oxidisable matters, and of the tannin as precipitated by Procter's and Hunt's methods. The difference between the results obtained by these two methods was attributed by Hunt to the precipitation of gallic acid by the excess of salt solution employed by Procter.

Material	Insoluble matter	Total extract	In terms of oxalic acid		
			Total oxidisable matters	Tannin Procter	Tannin Hunt
"Pure tannin"	......	......	135.76	122.44	121.93
English oak-bark	66.15	18.38	15.70	12.54	11.97
Canadian hemlock-bark	75.25	13.96	9.03	7.46	7.08
Larch-bark	60.80	20.64	8.20	7.17	6.15
Mangrove-bark	49.70	26.60	31.35	29.71	28.48
Alder-bark	68.00	19.36	8 27	6.15	5.73
Valonia	46.05	38.50	37.41	35.24	30.50
Myrobalans	......	42.80	48.23	38.43	38.00
Sumach	47 77	44.10	42.53	34.30	31.46
Turkish blue galls	36.35	48.40	73.38	65.83	59.96
Aleppo galls	14.32	68.80	98 85	87.82	83.05
Wild galls	54 17	31.70	26.21	18.75	16.56
Divi-divi	29.90	54.38	66.68	62.62	61.22
Pomegranate rind	49 50	41.00	27.58	24.18	23.12
Tormentil root	67 95	19.70	22.27	20.98	20.68
Rhatany root	66 00	18.80	22.27	20.15	19.30
Pure indian tea	53.40	34.46	23.06	18.65	17.40
Pure China tea	62 60	24 50	18.03	14.21	14.09
Cutch	4.75	61.60	57.65	51.95	44.24
Gum kino	1.00	79.30	66.39	59.55	51.55
Hemlock extract	......	48.78	35.16	33.17	30.98
Oak-wood extract	......	37.78	33.49	26.90	23.86
Chestnut extract	......	50.28	39.77	32.63	28.88
Quebracho extract	......	49.00	48.22	44.45	40.84
Tan-liquor (sp. gr. 1.030)	......	6 01	4.84	3.14	2.10
Spent liquor (sp. gr. 1.0165)	......	3.10	1.40	0.37	0.25

Hunt stated in the same paper that treatment with gelatin and salt does not remove all that is of tanning value from solutions of gambier and allied materials; hence he recommended the removal of the tannin in such cases by means of purified skin-shavings. These he added in the proportion of 5 grm. to 100 c.c. of a 1% gambier solution, and after 12 hours filtered and titrated the filtrate with permanganate in the usual way. The following results were obtained:

	Insoluble matter	Total extract	In terms of oxalic acid	
			Total oxidisable matter	Absorbed by skin
Cube gambier	5.31	74.40	70.12	51.07
Sarawak gambier	3.67	70.70	63.13	47.09
Bale gambier	1.40	63.54	56.00	43.70

The permanganate process of estimating tannin was submitted some years ago to examination by a commission of German chemists. After reviewing earlier methods they recommended the following modifications of the permanganate process for general adoption: 1. That the *permanganate* solution contain 10 grm. of $KMnO_4$ in 6000 c.c. 2. That the *indigo-solution* should be made by dissolving 30 grm. of air-dry sodium sulphindigotate in 3000 c.c. of dilute sulphuric acid (1:3), 3000 c.c. of water being added, and the whole shaken till dissolved, and the liquid filtered. Of this solution 20 c.c. in 750 c.c. of water should be used in each titration, and should reduce about 10.7 c.c. of the permanganate solution. 3. *Hide powder* was substituted for the ordinary gelatin solution, and was to be light coloured and in a fine woolly state of division, yielding to cold water no substance capable of reducing permanganate.

Instead of adding the permanganate solution drop by drop, the commission recommended (with very doubtful advantage) that an addition be made of 1 c.c. at a time, and the mixture vigorously stirred for 5 to 10 seconds after each addition. As soon as the liquid has become bright green, 2 or 3 drops at a time should be cautiously added with stirring, till the liquid is pure yellow. The results obtained by the "1 c.c. method" differ considerably from those obtained by the ordinary or "drop method" which was that employed by Neubauer and Oser for the estimation of the reduction co-efficients of tannins. It has, however, been shown by Procter, (*J. Soc. Chem. Ind.*, 1886, **5**, 79) that the results are more influenced by the rapidity of mixing than by the subsequent time of standing, and that the 1 c.c. method, while it gives a higher consumption of permanganate than the drop method, is more affected by variations in stirring.

Procter points out that the limit of the action is not a complete oxidation of the organic matter, but only a partial one of the bodies more readily oxidisable than indigo; and that hence towards the end of

the operation, when little indigo remains, the permanganate is partly consumed in further oxidising the products of the normal reaction; and that this is least the case when the permanganate is added slowly and rapidly mixed with the liquid, so as to bring it into immediate contact with the remaining indigo. Procter obtained very uniform results by the use of a stirrer consisting of a perforated porcelain disc, which was worked up and down in the beaker by means of an attached glass rod. He used a capillary jet to the point of the burette, allowing the permanganate to run in steadily throughout the titration.

E. B. (*Zeit. anal. Chem.*, 1886, **26**, 680) suggested the use of ferric acetate instead of gelatin for precipitating tannin. The process has been tried on gall tannin, and F. Gantter (*J. Chem. Soc.*, 1888, **54**, 540) does not confirm its accuracy in this case.

Other investigators have recommended the use of an ammoniacal solution of copper for removing the tannin. This is not capable of universal application. *Sumach* may be precipitated by ammonia and cupric acetate, and the tannin estimated by titrating the solution by permanganate and indigo before and after the treatment.

By this process, I. Macagno (*Chem. News*, 1880, **41**, 63) found that the upper side of sumach leaves was considerably richer in tannin than the lower, the proportion in old leaves being less than in young. The results varied from 8.77% of tannin in the lower side of old, to 25.82% in the upper side of young leaves.

It has been shown by Meyer (*Chem. Zeit.*, 1890, **14**, 1202) that the precipitation with copper acetate yields excellent results if the precipitation takes place in a hot solution, and washing with hot water follows immediately. The precipitate is dried at 110°, weighed, and ignited to CuO. From the weight of the total precipitate, four-fifths of the weight of the resulting copper oxide is deducted which gives the total tannin. This ignition might be saved by estimating the amount of copper required volumetrically and using this same amount in the precipitation.

N. H. Darton (*J. Amer. Chem. Soc.*, 1882, **4**, 4) employed copper ammonio-sulphate in the following manner: 20 grm. of *hemlock-bark*, or an equivalent amount of other tannin material, are extracted first with cold and then with several quantities of boiling water. The mixed infusions are treated with 25 c.c. of dilute sulphuric acid (1:10), the liquid filtered, and the filtrate rendered slightly

alkaline with ammonia, any precipitate being filtered off. A further quantity of 25 c.c. of dilute sulphuric acid is then added, and the liquid made up to 1 litre. 100 c.c. of this solution are treated with an equal measure of a solution of cupric sulphate (containing 1.25% of the salt), to which sufficient ammonia has been added to dissolve the precipitate first formed. The solution is passed through a dry filter, and a definite measure of the filtrate neutralised and titrated for "not tannin" with indigo and permanganate in the usual way. Procter stated that the preliminary treatment with acid and ammonia is unnecessary in the case of *valonia* (and probably in that of oak-bark), and that the process gave results practically identical with the then improved gelatin method, while it is much less troublesome. With *chestnut extract* the results are claimed to be satisfactory, provided the preliminary treatment be omitted, as this removes 75% of the matter precipitable by gelatin, and cutch behaves similarly. On the other hand, a sample of *larch extract*, which tanned well and showed 18% of tannin by the gelatin method, gave *no* precipitate with the ammonio-cupric solution. This peculiarity would allow of the estimation of valonia tannin in presence of larch tannin, and the same principle is utilised in other cases.

When applicable, the copper process has the advantage that the precipitate may be washed with a solution of ammonium carbonate, dried, and weighed;[1] or the precipitate may be ignited, the residue moistened with nitric acid, and re-ignited, and the cupric oxide weighed. Its weight, subtracted from the weight of the precipitate previously found, gives that of the tannin with which it was combined, or the latter may be found by multiplying the weight of CuO by 1.034. This factor probably applies only to gallotannin.

Dreaper (*Chem. News*, 1904, **90**, 111) gives the following details for his volumetric copper process. Standard solutions containing (1) copper sulphate equivalent to 0.05 grm. CuO per c.c. (2) 20 grm. lead acetate and 60 c.c. glacial acetic acid per litre, and (3) 50 grm. ammonium carbonate and 50 grm. sodium sulphite per litre are required.

(*a*) 50 c.c. of the tannin solution (containing 10 to 15 grm. per litre) are titrated with the copper solution after heating to 80° to 90° for a few minutes with excess of $CaCO_3$ (about 1 to 2 grm.) and cooling. The result in terms of CuO represents the total tannin and

[1] Dreaper recommends the precipitation in the presence of sodium sulphite to prevent oxidation (*Chem. News* 1904, **90**, 111).

gallic acid, and any "non-tannins" which precipitate copper salts, but not the non-tannins which may be carried down mechanically.

(*b*) A second 50 c.c. is taken and 10 c.c. of the lead solution added in the presence of barium sulphate. It is well shaken and after 5 minutes the lead tannin precipitate is filtered off through dry filter-paper, 0.5 grm. sodium sulphate (anhydrous) is added, and after 5 minutes the lead sulphate is filtered off. 40 c.c. of the filtrate are taken and titrated as in (*a*). The result gives the non-tannins precipitated by copper salts (gallic acid) and when subtracted from (*a*) gives the tannins also.

(*c*) 50 c.c. of tannin solution are taken and 25 c.c. of No. 3 solution added. The copper tannate formed on titrating this solution in the cold is free from gallic acid but only the tannins insoluble in ammonium carbonate are precipitated, so that a comparison with the results obtained in (*b*) indicates the amounts of total tannin, the two groups of tannins, and gallic acid and the non-tannins which may form compounds with copper, respectively. It is generally sufficient in practice to use only the lead separation which entirely removes all tannins but no gallic acid.

The end-point is obtained in all cases by removing a drop of the solution on a glass rod and pressing it into a doubled sheet of good filter-paper (*e. g.*, C. S. & S., No. 589, No. 3 brand). The under sheet will be then wetted with the filtered solution. A drop of ferrocyanide of potassium solution placed on this will show a pink coloration with an excess of copper. When testing the (*c*) solution in this way the ferrocyanide must be strongly acid with acetic acid, and the final result must be confirmed after an interval of 3 minutes, as the copper salt is precipitated slowly in this case. The process has been used to detect errors in the hide powder and "collin" processes. It gives the mordant value of any tannin and is not affected by any "non-tannins" or colouring matter carried down mechanically, or by free acid in the liquors. The presence of reducing sugars has no influence on the results.

C. W. Spiers (*J. Agric. Science*, 1914, **6**, 77) has used caseinogen as recommended by Nierenstein (*Chem. Zeit.*, 1911, **36**, 31) for the absorption of tannin from cider. Kahlbaum's pure caseinogen (nach Hammersten) was used, previously extracted 36 hours with ether.

It was found that for solutions of commercial "pure tannins" up to 0.5% concentration, detannising was completely effected by shak-

ing 50 c.c. with two quantities of one grm. of casein for 15 minutes. The liquid is filtered before the second addition of casein; and finally before titration through a barium sulphate filter, after which 5 c.c. are titrated as before.

The permanganate used was first standardised by means of Schering's tannin *leviss, puriss.* It was afterwards found that different samples of the same tannin gave somewhat different figures with the permanganate, since the commercial "pure tannins" are not homogeneous substances. A number of pure tannins were therefore titrated and the average value taken. Tannins purified by Nierenstein's and by Fischer's methods were included for comparison.

Table I contains the tannin value of the permanganate and the tannin content of the various samples used as found by detannising the solutions with casein. The water present in the tannin was estimated in all cases by drying to constant weight at 40° C. in a vacuum desiccator containing calcium chloride. Duplicate estiminations by this method usually differed by less than 0.05%. The dried samples were afterwards used for duplicate titrations with permanganate.

For convenience in standardising the permanganate the solution used was titrated against ammonium oxalate, which is easily obtained pure and is neither hygroscopic nor efflorescent. From this it was found, using the average value of the tannins as in Table I, that 1 grm. ammonium oxalate = 0.4648 grm. tannin.

Table II contains the results of analyses of various ciders; mostly of the bitter-sweet variety. The tannin content given by direct titration with permanganate is included for comparison. These, however, are probably somewhat lower than those found by Lloyd's method (*Report on Researches on Cider Making*, Board of Agriculture, 1903), since the small amount of indigo used by him would tend to make the tannin content appear larger by the inclusion of other oxidisable substances. The titration figures given here are those of the diluted permanganate. It should be noted that since the constitution of the cider-tannin is unknown, the amount of it present in the cider is expressed in terms of the tannin used to standardise the permanganate.

Although it was found that the strychnine method of Trotman and Hackford (*J. Soc. Chem. Ind.*, 1905, **24**, 1097) is not accurate in the case of gallotannin, the tannin in cider is quantitatively precipi-

TABLE I

Tannin	Tannin content %	Tannin value of permanganate grms. per c.c.
Schering's *leviss puriss.* Sample 1 undried	99.36	0.005450
Schering's *leviss puriss.* Sample 2 undried	95.66	0.005304
Schering's *leviss puriss.* Sample 2 dried	96.03	0.005270
Schering's *leviss puriss.* Sample 4 undried	96.61	0.005516
Schering's *leviss puriss.* Sample 4 dried	96.38	0.005319
Schering's *leviss puriss.* Sample 5 undried	94.56	0.005395
Schering's *leviss puriss.* Sample 5 dried	.....	0.005180
Merck's v. light; extra pure. Sample 1 dried	.....	0.005509
Merck's v. light; extra pure. Sample 1 undried	94.19	0.005399
Merck's v. light; extra pure. Sample 2 undried	91.24	0.005388
Merck's v. light; extra pure. Sample 2 dried	95.83	0.005993
Kahlbaum's Gerbsaure. Sample	....	....
Kahlbaum's Gerbsäure. Sample 1 dried	93.99	0.005591
Kahlbaum's Gerbsâure. Sample 2 undried	96.05	0.005526
Kahlbaum's Gerbsäure. Sample 2 dried	96.99	0.005540
Kahlbaum's Gerbsäure. Sample 3 undried	....	0.005454
Kahlbaum's Gerbsäure. Sample 3 dried	94.64	0.005623
Kahlbaum's Gerbsaure. Sample 5 undried	96.78	0.005463
Kahlbaum's Gerbsâure. Sample 5 dried	96.61	0.005921
Schuchardt's *leviss puriss.* Sample 1 dried	98.37	0.005421
Schuchardt's *leviss puriss.* Sample 1 undried	94.48	0.005318
Schuchardt's *leviss puriss.* Sample 2 dried	97.43	0.005620
Average, used for tannin content of ciders	....	0.005430
Schering's sample 3 purified by Nierenstein's method	....	0.004849
Schering's sample 3 purified by Nierenstein's method	....	0.004939
Schering's sample 3 purified by Nierenstein's method.	....	0.005130
Schering's sample 3 purified by Nierenstein's method	99.77	0.005216
Schering's sample 3 purified by Nierenstein's method	99.70	0.005117
Schering's sample 1 purified by Nierenstein's method	99.42	0.005170
Schering's sample 5 purified by the method of E. Fischer and Freudenberg	....	0.005554

TABLE II

Cider	S.G.	Total $KMnO_4$	Tannin $KMnO_4$	Tannin by direct titration	Tannin by casein method
Newton St. Cyres, bottled 7.2.12	1.035	18.26	14.65	0.38	0.31
Early Red Jersey (1) bottled 1.12.11	1.018	26.17	21.40	0.56	0.46
Early Red Jersey (2) bottled 1.12.11	1.007	27.35	21.83	0.59	0.47
Research Station Dabinett, bottled 15.1.12	1.028	11.48	9.09	0.24	0.19
Frederick B, bottled, 7.2.12	1.097	7.06	7.68	0.14	0.10
Twistbody Jersey (1) bottled 16.1.12	1.037	31.05	29.10	0.67	0.50
Twistbody Jersey (2) bottled 16.1.12	1.033	30.87	24.58	0.65	0.52
Sam's Crab, bottled, 20.1.12	1.031	7.70	5.80	0.16	0.12
Wallis' Red, bottled, 6.2.12	1.025	8.85	8.55	0.10	0.18
Farmer's Friend, bottled 5.1.12	1.029	4.21	2.61	0.00	0.05
Glastonbury Jersey, bottled, 15.2.12	1.030	27.84	22.09	0.59	0.64
Neverblight, bottled, 12.2.12	1.018	12.37	9.66	0.26	0.20
Farmer's Friend bottled, 5.1.12	1.019	2.87	1.76	0.06	0.04
Ashton White bottled 1912	1.001	12.61	10.02	0.27	0.21
Neverblight bottled, 15.2.12	1.018	12.12	9.44	0.26	0.20
Sweet Alford, sick, filtered 1	1.035	8.57	6.04	0.18	0.12
Newton St. Cyres, sick, filtered 1	1.023	12.85	10.50	0.26	0.22

tated by strychnine after careful neutralisation. This is shown by the fact that there is a parallelism between the results obtained by this method and by the permanganate titration method; although in the absence of a method of quantitative precipitation of a standard gallotannin and strychnine compound, the strychnine results cannot

be expressed as gallotannin comparably with those of permanganate titration.

TANNIN ESTIMATION BY THE HIDE POWDER GRAVIMETRIC METHOD

The official methods of the International Association of Leather Trades' Chemists, of the Society of Leather Trades' Chemists, and of the American Association of Leather Trades' Chemists are given later, but it is necessary here to explain a few details more fully to give reasons for the exact method prescribed and to discuss the various suggested modifications.

An infusion of the tannin material, or a solution of extract, of such a strength as to contain 0.35 to 0.45 grm. of tannin matter per 100 c.c. (or as nearly as possible 0.4 grm.) is prepared as variations from the prescribed dilution are specially apt to lead to errors with materials like quebracho, which contain much "difficultly soluble" tannin.

The following table gives the approximate quantities required:

THE APPROXIMATE AMOUNT OF DIFFERENT MATERIALS TO BE WEIGHED OUT FOR ANALYSIS TO MAKE UP ONE LITRE OF SOLUTION

Barks, etc.	Grammes	Extracts	Grammes
Algarobilla	8–9	Oak wood, sp. gr. 1.2 or over	15
Canaigre	15–18	Chestnut (liquid)	14
Divi-divi	9	Chestnut (solid)	7
Hemlock bark	32–36	Quebracho (solid)	6
Mimosa bark	11	Quebracho (liquid)	9–13
Myrobalans	15	Mimosa D	10–12
Oak bark	30–36	Gambier (block)	10
Oak wood	50–100	Gambier (cube)	7
Quebracho wood	20–22	Mangrove (liquid)	9
Sumach	15–16	Mangrove (solid)	7
Pistacia lentiscus	20–22	Cutch	7
Pine bark	32	Myrobalans (liquid)	16
Willow bark	36	Hemlock	10–14
Chestnut wood	45	Pine bark	16
Mangrove bark	10		
Valonia	14–15		
Valonia beard	10–11		
Spent tans	50–100		

Estimation of "Total Dry Matter."—It is necessary for the calculation of analyses to estimate the amount of moisture present in the sample, since this varies in solid materials to a considerable

extent with atmospheric conditions, and in extracts according to the degree to which they have been evaporated. The total weight of substance taken, less the moisture, gives, of course, the "total dry matter." The drying, however, presents difficulties in some cases. On the one hand, many tannins are easily alterable by heat, parting with water of combination and yielding anhydrides, and on the other hand, hygroscopic moisture is obstinately retained. Thus different temperatures and different methods yield varying amounts of moisture, and it is often impossible to define the point where hygroscopic water is driven off, and the loss of chemically combined water begins. A standard method would be to dry at ordinary temperature, and preferably *in vacuo*, over concentrated sulphuric acid, but this is too slow for practical requirements. In dealing with dry materials, therefore, it is better in every case, to work with as small a quantity as is permissible, taking into account the accuracy of the balance employed. As it is not possible in the analysis of the tanning materials to obtain an accuracy greater than 0.1 per cent. or one part per thousand, 1 grm. is quite sufficient with a balance weighing to milligrams, and with balances of greater accuracy, proportionately smaller quantities may be employed. In the case of liquid and pasty extracts, 1 grm. should never be exceeded, and if possible it is better to work with smaller quantities. The only exceptions to this rule are such materials as cannot readily be accurately sampled in small quantities, and in this case it may be necessary to weigh out larger quantities even at the expense of additional time and trouble, or to dissolve and dry an aliquot part of the solution as described below. The drying of solid materials is accomplished in basins or other vessels which are preferably fitted with a cover to lessen absorption of moisture during weighing, though with rapid weighing this is not essential. The drying of liquid and pasty extracts is more difficult, and is best done in the flat-bottomed shallow basins which are used for the subsequent estimations, and which should be 7 or 8 cm. in diameter and capable of holding at least 50 c.c. In such basins the extract spreads itself to a thin layer; whilst, if the basins are rounded, it collects at the bottom and dries on its surface, forming a coherent coating from which the moisture of the interior of the mass escapes with great difficulty. In cases where extracts are solid or too thick and pasty to spread themselves without assistance, the addition of

20 or 25 c.c. of water, in which the extract is dissolved on the water-bath in the basin, will considerably facilitate drying. The author proposed the use of alcohol for the same purpose, which certainly leads to rapid drying, but on the whole he is of the opinion that water is to be preferred. Where extracts will dissolve in water to a turbid solution which can be thoroughly mixed by shaking, the best method of estimation of both water and dry matter is to mix the liquid thoroughly, after making up to the litre (or 2 litres where it is desirable to weigh a larger sample), and before filtration, and to pipette 50 c.c. into a basin as usual. The residue obtained in this way × 20, or the sum of duplicate estimation × 10, is obviously the dry matter contained in the substance weighed out for analysis, and the difference between this and the original weight is the moisture; whilst the difference between the "dry matter" and the "total soluble" obtained by evaporation after filtration is the matter "insoluble at 15° C."

Drying Apparatus.—The German Section of the I. A. L. T. C., has selected the Moeslinger drying oven, which is simply a water-jacketed oven with small compartments, the interior of which cannot reach a temperature exceeding 95°–98° C. In this apparatus drying is, in the author's opinion, much too slow, at least 8 or 9 hours being required with many materials to obtain approximately constant weight. This length of time is objectionable not only on account of the delay, but because in most analyses, oxidation, leading to a gain of weight, goes on at the same time as drying; so that in some cases the sample begins to gain weight instead of remaining constant, and even the lowest weight found must be too high, as oxidation no doubt occurs more or less during the entire drying. The American Leather Chemists' Association have officially adopted Reed's Combined Evaporator and Dryer (*J. A. L. C. A.*, 1906, 32; see also *Collegium*, 1907, 140) which seems open to the same objections. In Procter's opinion a preferable method is the use of an air-oven heated to a temperature of 100°–105°, in which the drying is much more rapid, while the danger of oxidation is probably not increased. Great care, however, is required even in ovens with automatic regulation of temperature that 105° is not exceeded *in any part of the oven;* and that the vessels, especially if of metal, do not come into contact with the heated walls. There can be little doubt that the vacuum drying oven is the most perfect means

for drying tannin residues, accomplishing the work in two or three hours with as little exposure to oxidation as possible. There seems no reason why the vacuum-oven should be used at a temperature below 100°, as from its expense it cannot become the ordinary means of drying in small laboratories, which must dry at about 100°, and greater differences are likely to arise between this and working at a temperature at 60° or 70° than would occur by drying at 100° for the shorter period required by *in vacuo*. After drying for a sufficient length of time the sample is cooled in a desiccator, and rapidly weighed; it is then returned to the oven for half an hour or more, and the process repeated, placing the weight previously found upon the scale, so that the weighing can be almost instantaneous. If the loss of weight exceeds 2 mg. the sample should again be returned to the oven and the process repeated. If larger quantities than 1 grm. are being dried, a somewhat larger margin may be allowed.

Filtering.—A very frequent source of discrepancy in tannin analysis arises from the estimation of the *soluble matter*. Filter candles and filter papers absorb some of the soluble matter. It is difficult to estimate when a solution is "optically clear" as stipulated in the official methods, and when solutions which contain a large amount of soluble matter are filtered the pores of the filter paper or candle become lessened in size and *filtration is changed into ultra-filtration.* Brodetsky (*J. Soc. Leather Trad. Chem.*, 1922, **6**, 442) has made a mathematical study of the question, and has published the following summary of his results: "It is suggested that the soluble matter of tannin solutions should be estimated by allowing the insoluble particles to settle out and then evaporating 50 c.c. from the upper layer of liquor. This includes as soluble matter particles below a certain size, but eliminates the uncertainties of filtration and dispenses with judgments of optical clearness."

F. C. Thompson (*J. Soc. Leather Trad. Chem.*, 1922, **6**, 427) has given shape to Brodetsky's mathematical considerations and has made the following recommendations: The tannin infusion is poured into a glass cylinder about 5 cm. in diameter to a height of exactly 20 cm. A mark is made on the wall of the cylinder exactly 5 cm. below the surface of the liquor. The solution is allowed to stand exactly 2 hours, during which time the heavier insoluble particles settle out, leaving only very finely divided matter in suspension. 50 c.c. of the settled liquor are withdrawn from the liquor

above the mark, and this is used for the estimation of the soluble matter. This method obviates the errors due to different judgments on "optical clearness." Filtration with its various difficulties and uncertainties, is dispensed with. The time involved is limited, and there is no loss of time through slow filtration.

Estimation of "Total Soluble" Matter.—50 c.c. of the solution filtered by one of the methods described in the previous section, are measured with a pipette into a weighed glass or porcelain basin, as described in the previous paragraph, and evaporated to dryness on a water-bath. It is then dried eight hours, or overnight, in a water-oven at 100°, or, preferably, three hours in an air-oven heated to 100°–105°, or two hours in a vacuum-oven at 100°, cooled in the desiccator and weighed till constant as before. About three hours in the vacuum-oven and five hours in the air-oven are generally sufficient. Ultimately the residue may begin to gain weight from oxidation, and absolute constancy cannot always be obtained. In case of gain being noticed, the minimum observed weight must be taken, and this itself will obviously tend to be in excess of the truth, either from oxidation or imperfect drying, or both. The weight obtained after deducting that of the basin is that of the soluble matter of $1/20$ part of the substance originally weighed out, and hence the percentage is readily obtained by dividing double the weight of the residue in mg., or the sum of two duplicate estimations, by the weight in grm. of substances employed.

Platinum, nickel, or aluminium basins may be substituted for porcelain, with some gain in rapidity of evaporation. Platinum is important when the ash of the residue must be estimated, as in the analysis of old liquors, and but for its cost is most advantageous; nickel is less fragile than porcelain, but not suitable for ignitions, and aluminium is not to be recommended unless lightness is of great moment, as it is often attacked by the liquors to a slight extent; and this is specially apt to occur with the impure commercial aluminium used for cooking utensils. If it is used, care must be taken to prevent contact with the copper water-bath which sets up galvanic action and increases oxidation.

It is important that dry calcium chloride, or some other good water-absorbent should be employed in the desiccator, as the dry residues are very hygroscopic.

Estimation of "Soluble Non-tanning Matter.—Up to June 1907, the official I. A. L. T. C. method of detannisation was by the hide-powder filter. The siphon-filter generally employed is of a bell-shape, of a total length of 7 cm. with a diameter of 3 cm. in the cylindrical part, and 1.8 cm. in the neck, and resembles a bottle from which the bottom has been removed. Into the neck of this is fitted an indiarubber cork, with a piece of fine glass tubing, about 30 cm. long, projecting slightly through the cork, and bent into the form of a siphon. The short limb of this within the bottle is plugged with a tuft of cotton or glass wool, and the bottle is uniformly filled with between 6.5 and 7.5 grm. of hide-powder. The satisfactory filling demands some practice, and depends much on the quality of the powders. If the powder is too tight, or swells too much on being wet, the filter refuses to run, or runs too slowly; if too loose, the liquid forms channels and is not properly detannised. With pure hide-powders there is a tendency for the liquid to pass up the sides of the glass, and it is best to pack up pretty firmly round the sides, leaving it as loose as possible in the centre; but this difficulty has been almost entirely overcome by the admixture of cellulose (not exceeding 20 per cent.) with the hide-powder, so that it can usually be packed firmly and evenly. The use of cellulose for this purpose was first proposed by F. Cerych, (*Gerber*, 1895, 241, cp. also *ibid.*, 1896, 62). When full, the powder is kept in place by a piece of muslin secured over the bottom of the bottle with an indiarubber band. If the powder does not entirely fill the bell, the vacant space may be occupied with purified cotton or glass wool. The packed filter is placed in a beaker or tumbler, with its open bottom resting on the bottom of the glass, and the tanning infusion is added very gradually, little by little, so as to wet the powder by capillary absorption. When the filter and tumbler are filled with liquid, the siphon is started by sucking with a piece of indiarubber tube, and the filtered liquid is allowed to drop into a gauged cylinder. The first 30 c.c. are placed on one side, and the next 60 collected, from which 50 c.c. are pipetted, and evaporated for non-tannins. This should be quite or nearly colourless, and if a little of the liquid is now allowed to drop from the siphon into the first 30 c.c. no cloudiness should be produced. The soluble matter of the hide-powder contained in the first 30 c.c. is in this way a much more delicate test for tannin than any gelatin solution. Should the filtrate not be perfectly free from

tannin, the entire operation must be repeated, a more dilute solution being used.

The time required to obtain the necessary quantity of filtrate with a well-filled filter does not exceed 1 hour after the hide-powder has been thoroughly wetted; a somewhat longer time is not injurious. As it is extremely difficult to regulate the rate of filtration by the tightness of packing, it is usually best to use a piece of rubber pipe and a pinchcock on the siphon to regulate the flow to a rate of one drop in two seconds, which will give about 90 c.c. per hour. Very slow filtration, extending over more than two hours, usually increases the hide-substance dissolved, and consequently the amount of nontannins found. Cerych shows that high temperature of the laboratory during filtration also increases the amount of dissolved hide-substance, and advises that the temperature should be kept below 20°, even if in hot weather artificial cooling has to be resorted to. The temperature also influences the amount of "insoluble reds."

The most serious objection to the filter is that it undoubtedly absorbs other substances, such as colouring matters and acids, in addition to the actual tannin present. This defect is necessarily common to some extent to all hide powder processes, but is specially marked with the filter, in which the completely detannised liquor passes through a layer of completely untanned powder. As the absorptive power of raw hide for non-tannins is much greater than that of leather, the composition of the filtrate is never constant, the absorption of non-tannins constantly decreasing from the beginning to the end of the operation. In some cases the absorption of non-tannins is not of very serious importance from a practical point of view, but with materials such as sumach, which contain a large proportion of gallic acid, the latter is almost entirely absorbed by the hide, and not being volatile, is of course reckoned as tanning matter. A similar error occurs with sour liquors containing lactic acid. Several methods of overcoming this have been suggested, but none has hitherto proved entirely successful. Weiss found that the addition of common salt to the liquor (*Gerber*, 1887, 139) greatly reduced, but did not overcome it, whilst the presence of salt interfered with the drying of the residues. Procter found that alcohol added to the liquor prevented the absorption of gallic acid, but, if used in any large quantity, also interfered with that of tannin. Meerkatz employed barium carbonate to neutralise the acids present in sour

liquors. Barium salts remain dissolved in the liquors, and are taken up at first by the hide filter, but after 300 c.c. have passed are said to be no longer absorbed. Apart from the difficulty of securing complete absorption of tannin after so much as 300 c.c. has passed through an ordinary hide filter, the gallic acid salts rapidly become partly insoluble by oxidation, and apparently tannin is also liable in some cases to be precipitated, so that few chemists have succeeded in carrying out the process satisfactorily. In addition, it has been observed by Procter that neutral salts of weak acids, such as borax and sulphites, paralyse the absorption of tanning matter by hide, which precludes the use of such salts for neutralising acids in liquors, and is not without bearing on Meerkatz's process. It has, however, been found that the use of chromed hide powder considerably diminishes, though it does not prevent, the absorption of acids, and it entirely overcomes the swelling which with ordinary hide-powder is very troublesome in the presence of acids, frequently causing the filter to choke and refuse to run. Some particulars as to the absorption of substances other than tannin by hide-powders are given, but it may be remarked that most natural colouring matters, and many artificial ones, are completely removed. Thus the total tannin and colouring matter of a logwood extract may be estimated readily in the same way as tanning matters.

A dry, lightly chromed hide powder has been prepared by Paessler (*Collegium*, 1906, 27, 412) which obviates many of the difficulties incident to the use of ordinary hide-powder in the filter-bell, being easier to prepare and to free from soluble hide substances, and not so liable to swell and choke the filter in the presence of acids, so that its results show distinctly better concordance. It has, however, the defect of absorbing in most cases somewhat more non-tannins than even the unchromed powders, and therefore gives results 1 to 2 per cent. higher in "tanning matters." On this account it has never been accepted as official by the International Association, though it was for a period adopted by the German section. Used for the "shake" method, while fresh, it gives results in fair accordance with wet chromed powders.

Calculation and Statement of Analyses.—It is usual in hide-powder analyses to state the constituents under the following headings: "Tanning matter absorbed by Hide," "Soluble non-tanning Matter," "Insoluble Matter at 15°," "Moisture." To obtain these, in

addition to the estimation of tanning matter just described, a moisture estimation is necessary. This must be carried out at the same temperature as is used for drying the evaporated residues, but in the ordinary water-oven, gambier and some other extracts take an inordinate time to dry. At most, 1 to 2 grm. need be employed, if a balance of ordinary delicacy be available, and in the case of liquid extracts and gambier, 1 grm. should not be exceeded.

The calculation is made as follows: The percentage of tanning matter is obtained by subtracting that of Soluble Non-tannins from that of Total Soluble. The Insoluble is similarly found by deducting the Total Soluble from the Total Dry Matter obtained in the moisture estimation. The four must necessarily add up to exactly 100 if the calculation is correct. If an extract is quite free from insoluble matter, it occasionally happens that the Total Soluble apparently exceeds the Total Dry Matter, in consequence of oxidation during drying. In this case, in which the difference should not at most exceed a few tenths per cent., it is best to neglect the Total Soluble, and use the Total Dry Matter in the calculation of the Tanning Matter.

It is often desirable to estimate ash and inorganic matter in the residue of the moisture estimation, but it should not be added in with the other constituents, as it is impossible to say whether it is derived from the soluble or insoluble portion, unless the residues of Total Soluble are also ignited. The ignition should be very gentle till all the carbon is destroyed, on account of a possible presence of fusible salts. In sumachs, ash is important as affording an index of the degree of "ventilation" or removal of sand, which is often ferruginous, and darkens the colour of the liquor and leather. The total ash should not much exceed 6 per cent. in a really pure sumach. The natural ash of the sumach may be practically removed from the siliceous sand, mostly of volcanic origin, by washing with dilute hydrochloric acid and again igniting. If limestone is present, it will of course go with the ash. Turnbull estimates sand and the heavier mechanical impurities by shaking a weighed quantity with commercial carbon tetrachloride in a pear-shaped separating funnel, with a wide-bore stopcock, when the sand settles to the bottom and can be decanted into a basin, dried, and gently ignited. Sumachs frequently contain among the sand particles of magnetic oxide of iron, which may be detected by sorting the dry sumach with

a magnet to the edge of which the dark metallic looking particles adhere, and on which they are easily seen with a lens. They may be distinguished from actual metallic iron by solution in a drop of dilute sulphuric acid, if necessary, on a glass slip under the microscope. The magnetic oxide dissolves without effervescence to a yellow solution, and the metallic iron to a colourless one with evolution of hydrogen. The total iron in the ash may be estimated colorimetrically. Barks and bark extracts usually contain a little manganese, which colours the ash green if fused. In extracts the quantity and character of the ash often give useful hints as to the methods of manufacture. Artificially solubilised extracts usually contain an increased quantity of soluble alkaline carbonates.

A rather considerable disadvantage in the use of wet chromed hide powder is that it will not keep for any length of time in a moist condition, and consequently has to be prepared from a good hide-powder as required, and that it is very difficult, if not impossible, to free the wet hide-powder completely from soluble salts and especially from sulphates before it has undergone some decomposition. To overcome this difficulty various attempts have been made to prepare a dry chromed hide powder, but with very varying success, owing to the conditions not being fully understood. In 1901, the Vienna Research Station adopted a method of preparing such a powder, which, while it gave somewhat higher results in non-tannins than the filter method, was other-wise satisfactory, and was much less affected by the presence of acids.

The great difficulty in the use of chromed hide-powder is that when thoroughly dried it absorbs water extremely slowly. It is, therefore, necessary to weigh out the quantity of the air-dry powder which is required for the estimations expected, and mix it with water to a thin paste, and allow it to soak for at least six hours (preferably overnight), 10 grm. of powder being allowed for each estimation. After soaking, it is freed as completely as possible from water by being squeezed in cheese-cloth, mixed with a fresh quantity of distilled water, and again squeezed out as tightly as possible and well mixed. The total weight should be about 30 grm. of wet for each 10 grm. of air-dried powder, containing about 70 per cent. of moisture, in the wet, and 10 per cent. in the air-dry state. With practice it is easy to get within 1 per cent. of the moisture stated in the wet powder. If too wet, mixing on filter paper will

rapidly reduce moisture. Accurate estimation of the moisture is unnecessary.

50 grm. of the wet powder are used for the detannisation of 150 c.c. of the tannin solution, which may be conducted in a wide-mouthed bottle, cylinder, or beaker (tumbler). Either shaking or maceration will give approximately equal results, but further experience is needed to determine the time of shaking exactly equivalent to one hour's maceration, which Kopecky regarded as the standard method. Longer maceration, or repeated filtering, will slightly increase absorption. The mixture is now poured on to a filter-paper in a funnel covered by a clock-glass and is allowed to run through, and *twice* returned to the funnel.

50 c.c. of the solution are evaporated as usual, and the result corrected by multiplication by $\frac{150 \times \textit{water of 30 grm.}}{50}$, the moisture being estimated on a portion of wet powder.

Some few dark-coloured materials, and especially some quebrachos and mangroves, of which the infusions cannot be detannised by the hide-filter without dilution, also require the use of 40 instead of 30 grm. of wet powder in the present method.

The method is equally adapted for the estimation of tannin in fresh materials and in sour liquors, being little affected by the presence of organic acids, and answers excellently while the powder is fresh, but it has unfortunately been found that its absorbency lessens on keeping. This appears to be a defect common to all chromed powders, though less obvious when the chroming is light, and is probably due to a gradual loss of moisture which the fibre will not re-absorb. It is to be hoped that Kopecky will succeed in so modifying his powder as to overcome this difficulty, since the use of a dry chromed powder is very convenient.

It may be taken as proved, that either chromed or unchromed hide powder in moderate excess will absorb the whole of the actual tannins contained in any solution of the prescribed strength; but it has already been pointed out that the so-called "tanning matters" absorbed contain many other things which are not truly tannins, or perhaps strictly even "tanning matters." It therefore becomes of importance to know what is actually absorbed under given conditions, and, though it would be difficult to make any really exhaustive investigation, the following will throw some light on the subject:

OFFICIAL METHOD OF TANNIN ANALYSIS OF THE SOCIETY OF LEATHER TRADES' CHEMISTS (1924)

This is the Official Method for Great Britain, France, Italy and Spain

GENERAL REGULATIONS

APPARATUS

Glassware.—Graduated flasks and pipettes shall be carefully verified and, if necessary, regraduated.

Desiccators.—Shall possess a tight-fitting cover and contain anhydrous calcium chloride. Not more than two basins shall be placed to cool in each desiccator.

Evaporation.—All evaporation shall be rapidly conducted at steam temperature in shallow flat-bottomed basins. Porcelain rings shall be used on the water or steam bath used for evaporation.

Drying Ovens.—Residues shall be dried in an oven of a uniform and constant temperature of 98.5–100°, water, steam, vacuum steam, and electric ovens being permissible. The last mentioned must be provided with a device for automatically regulating the temperature. Gas-heated air ovens must not be employed for drying residues.

Evaporating Basins.—Evaporating basins must be shallow, flat bottomed, and without sharp angles, not less than 6.5 c.m. in diameter. Porcelain basins may be used, these should be glazed inside and outside—but silver basins are preferable as they allow more rapid evaporation.

Balances.—Analytical balances, accurate to 0.2 mgrm. with 100 grm. load, shall always be employed for weighing residues.

Berkefeld Filter Candles.—Berkefeld filter candles shall be employed for the filtration of the tannin infusions. New candles should be soaked in changes of 10 per cent. hydrochloric acid for several days on the steam bath in order to free them from iron and other soluble matters. They are then thoroughly washed until free from acid, and dried on a draining board sheltered from dust.

Linen.—Linen cloths are to be used for washing the chromed hide powder and for the preliminary filtration of the detannised solution. The linen must be unbleached and sufficiently coarse to allow water to be easily squeezed through. Before use it should be well boiled in several changes of distilled water to free it from weighting matter.

Only distilled water may be used for this purpose, and on no account must soap be used for washing the cloths.

Filter Paper.—The filter paper to be employed for obtaining a clear filtrate of the detannised solution shall be 18.5 cm. in diameter. Schleicher and Schull No. 605 folded, or Munktell No. 1 F. Swedish paper may be used for this purpose.

Chemicals

Distilled water must comply with the following specifications:

(*a*) It must be free from sulphates and chlorides.

(*b*) The pH value must be between 5.0 and 6.0, that is, it should not yield a red colour with methyl red, nor a deep purple with brom-cresol purple (bromcresolsulphonephthalein).

(*c*) The residue after evaporation of 100 c.c. must not exceed 0.002 grm.

Kaolin.—The kaolin should be washed with hydrochloric acid, preferably by the analyst himself, in order to remove soluble matters, and afterwards washed with distilled water until free from acid. After this treatment, 1 grm. of the dried kaolin should be suspended in 100 c.c. of water and well shaken. This suspension is then tested with indicators, and should show a pH value between 4.0 and 6.0, that is, it should not give a red colour with Methyl Orange nor a deep purple colour with brom-cresol purple. I grm. of the purified kaolin shaken with 100 c.c. of N/100 acetic acid (0.6 grm. glacial acetic acid per litre) should leave less than 1 mg. of residue after filtration, evaporation and drying.

Basic Chromium Chloride Solution.—This is made by dissolving 100 grm. of $Cr_2Cl_3.6H_2O$ in a little distilled water to which is added *very slowly with constant stirring* a solution containing 30 grm. of pure anhydrous sodium carbonate dissolved in a small quantity of water and the solution heated on the water bath until all carbon dioxide is expelled. Cool and make up to 1 litre. If these instructions are carried out there should be no precipitate after making up to a litre with distilled water and well mixing. 1.3 c.c. of the stock solution is to be employed for chroming each lot of 6.5 grm. of dry hide powder. The chromium chloride employed should be chemically pure and correspond to the formula $Cr_2Cl_3.6H_2O$ and the stock solution prepared shall be tested for its basicity. It should corre-

spond to the formula $Cr_2Cl_3(OH)_3$ and have a basicity of 50 (new system).

Hide Powder.—The hide powder used shall be that passed as official by the International Hide Powder Committee, supplied in sealed tins and labelled (See appendix).

Reagents for Chlorides.—These reagents are employed for ascertaining if the chromed hide powder has been sufficiently washed; 10% solution of pure potassium chromate K_2CrO_4 and N/10 solution of silver nitrate $AgNO_3$ (17 grm. per litre).

Gelatin Salt Reagent.—1 grm. of leaf gelatin and 10 grm. pure sodium chloride are dissolved in 100 c.c. of distilled water, and the reaction is adjusted to approximately pH 4.7 by the addition of a little acetic acid. Such a solution should give a red colour with methyl red and a yellow with Methyl Orange. 2 c.c. toluene added to this solution will preserve it for a considerable time. In the preparation of this reagent the temperature shall not be allowed to exceed 60°.

Preparation of Samples for Analysis

Solid Tanning Materials (Woods, Barks, Fruits, etc.).—Woods, barks, and fruits, are ground in a suitable mill until they will pass through a sieve of five wires per linear centimetre. When from the fibrous nature of a solid tanning material it cannot be ground so as to pass entirely through the specified sieve, the finer and coarser portions must be separately weighed so as to estimate the proportions of fine and coarse material in the whole amount ground.

The quantity of material actually used for extraction must consist of fine and coarse material in the same proportions.

Any material giving finely divided matter (dust) on grinding must be dealt with in the same way, *i. e.*, the portion extracted must consist of dust and coarser material in the proportions existing in the whole ground sample.

Some materials lose moisture when submitted to the grinding process and it is advisable therefore to estimate the moisture both before and after grinding, and if any loss has taken place the results obtained on the dried sample should be calculated back to the original moisture.

Sumach.—Samples of ground sumach must be thoroughly mixed in a rotating churn jar before weighing out for extraction.

Solid Extracts.—Solid extracts shall be ground in a porcelain or agate mortar before weighing out. Certain solid extracts are of uneven moisture content and often cannot be pulverised and ground. The blocks should be broken up, weighed, and then allowed to dry in an air oven at 70° for some hours until they can be pulverised.

After this partial drying, the extract is again weighed and the loss of moisture ascertained. The material is then ground in a mortar and the results of analysis calculated to the original water content.

Pasty extracts such as block gambier should be cut up into small portions and treated in the same way.

Liquid Extracts.—Liquid Extracts should be thoroughly mixed in a shake machine to cause an even distribution of the deposit which is often present.

Viscous extracts should be heated to 45° in the water bath, well mixed, cooled to 17–18° and then weighed out at once. This heating must be mentioned on the report form.

Preparation of Infusion

Such a quantity of material shall be employed as will give a solution containing as nearly as possible 4 grm. of tanning matter absorbable by hide powder per litre and in any case not less than 3.75 nor more than 4.25 grm. In the event of the results of analysis showing a tannin strength outside these limits, the analysis shall be repeated.

All materials for analysis shall be weighed out on an analytical balance to an accuracy of 0.005 grm.

Liquid Extracts.—Liquid Extracts shall be weighed as rapidly as possible in a basin or beaker, washed with boiling distilled water into a litre flask, filled up to 900 c.c. with boiling distilled water, and rapidly cooled to 17–18° (*vide infra*). In the analysis of sumach and myrobalans extracts and synthetic tanning materials, distilled water at 70° must be used.

Solid Extracts.—Solid Extracts shall be weighed out in a basin or beaker and heated on the water bath with about 1½ times their weight of distilled water until no solid fragments remain, except fibrous matter in such materials as gambier.

Then proceed as with liquid extracts. Pasty extracts, such as block gambier, should be treated in similar manner.

Solid Tanning Materials.—Solid Tanning Materials ground as previously described are weighed out and extracted in the Procter

extractor with 500 c.c. of water at a temperature not exceeding 50° and the extraction continued with boiling water until the percolate amounts to 1 litre. The material must be allowed to soak in cold distilled water for not less than 12 or more than 18 hours (*e. g.*, overnight) before commencing percolation. The method of extraction adopted shall be that of continuous percolation, *i. e.*, a constant level of liquid shall be maintained in the extraction beaker throughout the process except that at the end of the extraction the beaker shall be allowed to drain empty. The extraction must proceed evenly throughout except that when the first 500 c.c. of extract have been collected the extraction is stopped until the temperature is raised as quickly as possible to 98–100°[1] and the extraction continued until 1 litre has been collected. The time of extraction shall be 3–4 hours. Any remaining solubles in the material must be neglected. A simple form of continuous percolation apparatus is described in the appendix.

Cooling.—The infusion so prepared is well mixed and rapidly cooled to 17–18° by immersion in water kept at about 15° after which the flask shall be accurately made up to the mark with distilled water, well mixed in a clean dry flask of about 1500 c.c. capacity and filtration at once proceeded with. Cooling by means of a stream of running cold water impinging directly on the sides of the flasks is to be avoided, owing to the danger of local chilling and precipitation of slightly soluble tannins.

Estimation of Moisture and of Total Solids

For any tanning material examined the sum of the moisture and total solids is 100% so that an estimation of one of these is sufficient.

In general, a direct determination of moisture shall be carried out on solid tanning materials and also on extracts such as gambier that do not yield a uniformly turbid solution.

Moisture.—About 1 grm. of the finely ground material is accurately weighed out in a squat wide-mouthed weighing bottle and dried between 98.5–100° in a water or steam oven, cooled in a desiccator for 20 minutes and weighed on the analytical balance as quickly as possible. It is then returned to the oven and afterwards dried until the weight is constant. If the weight of the

[1] The extraction of materials containing much starch, *e. g.*, canaigre, is to be carried out completely at 50°.

dried material is found to increase with longer drying, then the lowest weight found must be taken.

Total Solids.—Total Solids are estimated by evaporating to apparent dryness 50 c.c. of the well mixed and uniformly turbid infusion in the shallow flat-bottomed evaporating basins previously described, on a water-bath or combined water-bath-steam oven. The basins are subsequently dried, cooled in a desiccator and weighed as rapidly as possible to an accuracy of 0.2 mg. until constant weight is attained, as described above. The basins must not be wiped after removal from the desiccator.

Estimation of Solubles and Insolubles

The infusion shall be filtered, repeatedly if necessary, until "*optically clear*" both by reflected and transmitted light—that is, a bright object such as an electric light filament must be distinctly visible through at least 5 cm. thickness and a layer 1 cm. deep in a beaker, placed in a good light on black glass or black glazed paper, must appear dark and free from opalescence when viewed from above.

For the filtration, Berkefeld filter candles must be employed, and these must be clean and dry. 250 c.c. of the filtrate must be rejected and, after this, 50 c.c. are collected for evaporation as soon as the filtrate becomes optically clear. These 50 c.c. are evaporated to apparent dryness in the shallow flat-bottomed basins already specified, on a water-bath or combined water-bath and steam-oven. The basins are subsequently dried between 98.5° and 100°, cooled in desiccators for 20 minutes and weighed as rapidly as possible to an accuracy of 0.2 mg. until constant weight is attained, as previously described in the estimation of moisture and total solids.

Should the filtration become very slow owing to the formation of a slimy deposit on the candle, the latter may be cleaned by brushing with a soft brush (for example, a tooth brush) and some of the unfiltered tannin infusion.

Cleaning of Berkefeld Filter Candles

The candles, before use, should be treated as previously described to remove traces of iron and other soluble matters. Immediately after the completion of the analysis the candles must be cleaned by

brushing under a stream of water with a soft brush and then rinsed by aspirating warm distilled water at 70–75° C. through them until the filtrate is quite colourless. They must then be left to dry on a draining board sheltered from dust.

Sometimes the pores of the candles become clogged with a slimy deposit, especially after filtering such infusions as those of untreated quebracho extract, and the candles cannot be cleaned by simply washing with water. In these cases a few c.c. of ether must be added to the cold distilled water and the candles cleaned by drawing this liquid through them and finally finishing off the washing with warm distilled water until the filtrate is quite colourless.

Estimation of Non-tannins

The tannin infusion must be detannised by shaking with chromed hide powder until no turbidity or opalescence is produced in 10 c.c. of the clear solution by the addition of 2 drops of the gelatin and salt reagent.

A multiple of that quantity of hide powder containing 6.5 gm. of dry matter, according to the number of analyses to be made, is wet with about 10–12 times its weight of distilled water. To this is added 1.3 c.c. of the stock basic chromic chloride solution for each 6.5 grm. of dry hide powder and the whole churned for 1 hour in a glass bottle fitted with a rubber bung, at 60–80 revolutions per minute after which the powder is immediately washed as follows:

Washing of the Chromed Hide Powder.—The contents of the shake bottle are poured on to a Buchner funnel of such size as will conveniently hold the whole of the hide powder being chromed and fitted with a close fitting circular piece of cloth placed on the bottom of the funnel. The funnel is fitted into a filter flask connected in its turn to a vacuum pump. (A funnel 15 cm. in diameter will suffice for the equivalent of 26 gm. of dry hide powder.)

Any portions of the hide powder remaining in the shake bottle are rinsed into the funnel. The vacuum pump is then applied to suck off the liquid and the hide powder pressed down hard with a spatula. As much liquid as possible should be removed after each washing in order to free the powder quickly from soluble chlorides. The powder is washed with successive quantities of distilled water, taking care to break up the cake of powder each time and to stir well before sucking off the wash waters. Four or five washings

should usually be sufficient to reduce the sodium chloride content to less than 1 mgm. per 50 c.c. of washings at which stage 1 drop of 10 per cent. potassium chromate solution and 4 drops of N/10 silver nitrate solution should yield a brick red colour with the last 50 c.c. of the washings.

The hide powder is then squeezed, and as much water as possible removed (conveniently by means of the rubber bung) so that the weight of wet hide powder corresponding to 6.5 grm. of dry powder is less than 26.5 grm. (The hide powder shall contain between 70 and 75 per cent. of water.) The wet powder is removed from the Buchner funnel and from the filter cloth by means of a spatula, the caked mass being broken up into small fragments and the last traces separated from the cloth by folding the four corners together and beating smartly on a *clean* bench to collect the powder in the middle of the cloth. The powder is weighed to approximately 0.05 grm., the quantity "q" representing 6.5 grm. dry powder calculated and the whole lot of chromed hide powder divided into smaller lots, each representing 6.5 grm. dry hide powder. Each lot is then added to 100 c.c. of the unfiltered tannin infusion in a shake bottle together with $(26.5 - q)$ c.c. of distilled water. The whole is shaken smartly for an instant after corking and placed in the shaking machine rotating at 60–80 revolutions per minute for 15 minutes. The contents of the bottle are poured on to linen in a funnel, and the detannised liquid pressed out with a spatula. The filtrate is stirred up with 1 grm. of purified kaolin and filtered until clear through a folded filter paper as already specified. The non-tannin filtrate must be tested and must give no turbidity with 1 or 2 drops of the gelatin-salt reagent.

60 c.c. of the non-tannin filtrate shall be evaporated and reckoned as 50 c.c., or the residue from the evaporation of 50 c.c. is multiplied by $6/5$. Evaporation, drying, and weighing until constant weight is attained, shall be carried out as previously described for the estimation of total solids and total solubles.

Tanning matter absorbable by hide powder is the difference between the percentages of total solubles and non-tannins.

Insoluble Matter.—Insoluble matter is the difference between the percentages of total solids and total solubles or between 100 per cent. and the sum of the percentages of moisture and soluble matter in the cases of solid tanning materials where moisture is directly estimated.

Specific Gravity.—Specific gravity shall be determined by the specific gravity bottle or pyknometer, keeping the temperature as close as possible to 15° C.

Analysis of Used Liquors and Spent Tanning Materials

The analysis of used liquors and spent tans shall be carried out by the same methods as laid down for fresh tanning materials.

Used liquors shall be diluted until the specific gravity is 1.007–1.008 (7–8° Barkometer). If the liquors are already weaker than this, the amount of dry hide powder used in the detannisation shall be 1 gm. for every degree of Barkometer or for every thousandth of a unit of specific gravity greater than 1.000.

The specific gravity of used liquors shall be reported.

The amount of spent tanning materials taken for analysis shall be such as to yield an infusion containing between 3.5 and 4.5 grm. of tannin matter per litre if possible, but in no case must the concentration of total solids exceed 10 grm. per litre. The quantity of dry hide powder to be taken for analysis shall be 6.5 grm.

Analytical results for spent materials shall be calculated on the dry matter basis, and for spent or used liquors in terms of grams per 100 c.c.

Accuracy of the Method

All analyses shall be the average result of duplicate estimations. The weights of residues shall in all cases agree within 2 mg., so that the absolute error in the tannin content is not more than 2 per cent. Thus for liquid extracts containing 30 per cent. of tannin the duplicate results for percentage of tannin shall agree within 0.6 per cent., and for solid extracts of 60 per cent. tannin content within 1.2 per cent. The analysis shall be repeated if necessary until such agreement is reached and it must be clearly stated on the report that the results are the means of such estimations.

Where analyses are carried out by different chemists on the same extract or tanning material, their results should not differ by more than 3.33 per cent. of the total tannin content. The permissible differences are therefore as follows:

Tannin content, %	Difference allowed, %
25	0.8
30	1.0
40	1.3
50	1.7
60	2.0
65	2.2

Conclusion

All analyses carried out by members of the Society shall be performed in strict accordance with the foregoing instructions and the report form must state that "the analysis has been made by the Official Barcelona Conference Method of the Society of Leather Trades' Chemists."

APPENDIX I

Hide Powder.—Tanning analyses carried out by members of the Society of Leather Trades' Chemists shall be performed with the batch of hide powder recognised as official by the International Hide Powder Committee. The adoption of any official batch of hide powder will be notified in the Journal of the Society.

Analyses carried out with a superseded batch of hide powder will not be considered as official analyses.

Characteristics of Hide Powder.—Hide powder shall be of a woolly and fibrous texture. 6.5 grm. of the dry powder suspended in 100 c.c. of distilled water shall not require more than 5.0 c.c. nor less than 2.5 c.c. of N/10 NaOH to produce a permanent pink with phenolphthalein.

If the acidity of the powder does not fall within these limits it must be corrected by soaking the powder before chroming for 20 minutes in 10–12 times its weight of distilled water, to which the requisite amount of standard acid or alkali has been added to bring the acidity to the nearer of the prescribed limits.

Hide powders that possess an acidity corresponding to a value between 2.5 and 5.0 c.c. of N/10 NaOH per 6.5 grm. of dry powder shall not be corrected.

Recent batches of hide powder have not required any correction for their acidities.

Hide powder must not swell in the chroming operation to such an extent as to render difficult the necessary squeezing to 70–75 per cent. of water, and must be sufficiently free from soluble organic matter to render it possible in the ordinary washing to reduce the solubles in a blank experiment with distilled water below 5 mgm. per 100 c.c.

The following estimations are the official methods employed by the International Hide Powder Committee to control different batches of hide powder:

Moisture.—A stoppered weighing bottle 1¼ inches in diameter is heated for 15 minutes at 100° in the steam oven with the stopper out, cooled in a desiccator for 20 minutes, with the stopper in, and then weighed; (W_1).

After allowing it to stand for 5 minutes in the balance case, it is reweighed; (W_2). It is then immediately filled with hide powder, about 2 grams being added, the packing being as loose as possible. The stopper is at once replaced and the bottle and contents weighed; (W_3).

The bottle and contents, with the stopper out, are then dried for 3 hours at 100°, preferably in a steam oven. The stopper is then replaced, and the bottle and contents cooled in a desiccator for 30 minutes and again weighed. The drying is continued for another hour, to see if the weight is constant, and if not, continued still longer until a constant weight is obtained; (W_4).

The percentage of moisture in the powder will then be

$$\frac{100(W_3 - W_2) - (W_4 - W_1)}{W_3 - W_2}.$$

A duplicate experiment should be done also, taking the hide powder from a different part of the bulk sample.

Acidity.—The quantity (Q) of the air dry hide powder which corresponds to 6.5 grams dry hide powder is shaken for about 10 minutes in an ordinary shake bottle with 100 c.c. distilled water, shaking the mixture occasionally by hand. After adding 1 c.c. of a 1 per cent. alcoholic solution of phenolphthalein, the mixture is titrated with N/10 NaOH until a permanent pink colour is obtained. Results are stated in c.c. N/10 NaOH required.

A pink coloration lasting for five minutes is to be regarded as permanent.

Soluble Matter.—The quantity (Q) of hide powder, used for each analysis, is placed in an ordinary shake bottle with ($126.5 - Q$) c.c. of distilled water, and churned slowly for one hour. It is then filtered through linen, and through filter paper, with the assistance of about 1 gram of kaolin, and 60 c.c. filtrate are evaporated to dryness and dried to constant weight. The weight of the residue multiplied by two gives the "soluble matter in 6.5 grm. dry hide powder."

Blank Test.—This should be carried out in exactly the same way as in an official detannisation, but 100 c.c. of distilled water are employed instead of 100 c.c. of tan infusion. Two tests should be carried out side by side, like duplicate analyses, *i. e.*, the hide powder should be chromed, washed and squeezed as a whole, but separated for the two analyses: 60 c.c. are evaporated in each case, and the residues added together and reported as one result—"Residue of blank test per 6.5 grm. of dry hide powder."

Ash and Alkalinity of the Ash.—The ash content of the hide powder is estimated by igniting 5 grm. of the powder to constant weight in a platinum crucible or dish, employing a Teclu or Meker burner.

The alkalinity of the ash is estimated by treating the ash with a known excess of N/10 H_2SO_4, and titrating the excess with N/10 NaOH and Methyl Orange.

Comparative Non-tannin Estimations.—Comparative non-tannin estimations should be carried out to compare one batch of hide powder with another, that is, to replace it, if satisfactory.

APPENDIX II

Procter Extractor.—This apparatus consists of a beaker of suitable size for the quantity of material taken, which is placed in a water-bath. A thistle funnel of which the stem is bent twice at right angles is covered with muslin or silk gauze and placed head downwards in the beaker, and about 20 grm. of silver-sand (purified with HCl) and the weighed tanning material are added.

After the necessary maceration (preferably overnight) the liquid is sucked over, and the flow regulated with a screw clip. The glass and rubber siphon tube is usually about 10 inches long.

As the liquid siphons, it is replaced by distilled water of the same temperature as the water-bath. A simple device permitting continuous percolation has been described by L. Sheard (*Collegium*, 1908, 277); it consists of a reservoir of distilled water, placed on a

shelf above the extraction bath, thus allowing distilled water to replace the liquid that has percolated over from the beaker. By means of glass tubing bent into a worm to surround the beaker, and which is immersed in the water-bath, the distilled water can be heated to the temperature of the bath, and a screw clip will regulate the flow. This clip should be closed when the sum of the volumes of liquid in the extraction beaker and in the collecting flask is approximately 1 litre. The contents of the beaker are then siphoned until 1 litre of percolate has been obtained.

APPENDIX III

Approximate Quantities of Materials to Be Taken for Analysis in Grams per Litre

SOLID TANNING MATERIALS (WOODS, BARKS, FRUITS, LEAVES, ETC.)

Canaigre	15–18	Myrobalans (pulp only)	8–10
Chestnut wood (fresh)	50–55	Myrobalans (whole nuts)	12–14
Chestnut wood (dry)	38–42	Valonia (whole cups)	14–15
Quebracho wood	19–21	Valonia beard (trynacks)	9–10
Hemlock bark	32–36	Divi-divi, algarobilla, teri and gonakie	10–12
Mimosa bark	10–14	Sumach	15–16
Oak bark	35–45	Spent tans	50–80
Mangrove bark	10–12		
Pine bark	30–35		
Solid extracts:			
Chestnut	6–7	Sumach	6–7
Mangrove	6	Cutch	10
Quebracho (natural)	6	Gambier, cube	12–14
Mimosa bark	6–7	Gambier, block	14–16
Liquid extracts:			
Chestnut	13	Myrobalans	16
Quebracho, sulphited	12	Hemlock	11–13
Mimosa	11–13	Sulphite cellulose (wood-pulp)	16–18
Oak wood	16		

Official Methods for Sampling Tanning Materials (1924)

General.—As the divergence between the results obtained by different chemists has, in many cases, been traced to the inefficient manner of sampling, the following regulations have been drawn up.

All tanning materials contain moisture in different proportions depending on the nature of the material and also on climatic conditions, so that sampling should be carried out as quickly as is consistent with thoroughness, in order to avoid loss of moisture.

Immediately after taking the samples, they should be put into clean and dry **glass bottles,** well corked, sealed and labelled.

Four samples should, in general, be taken, one each for the buyer, the seller, the independent analyst, and the fourth as a reserve in case one of the other samples is lost or damaged.

Number of Barrels or Packages to Be Sampled.—The number of packages or barrels to be sampled out of any given lot of tanning material, irrespective of whether it is composed of bark, solid or liquid extract, shall be seven-tenths of the square root of the total number of packages, etc., in the lot, fractional parts of a package to count as a whole one, as illustrated in the following table:

Number of packages in total consignment "x"	$0.7 \times \sqrt{x}$	Number of packages to be sampled
10	2.21	3
20	3.12	4
50	4.95	5
100	7.0	7
200	9.9	10
400	14.0	14
1000	22.1	23
2000	31.2	32

Barks and Wood in Sticks.—Barks and other materials in bundles shall be sampled by cutting a short section about 1½ inches long from the middle of the calculated number of bundles. The cutting through of the samples may be made with a saw or some other suitable instrument such as a pair of strong shears. The sub-samples so taken shall be mixed together and the bulk reduced by quartering to the desired size.

The weight of each sample shall be at least 1 kilogram.

Wood in Logs.—The calculated number of logs is set aside, selecting logs of varying size so as to represent the bulk of the consignment, and they are then sawn through. The whole of the sawdust so obtained is well mixed and 500 grm. taken for each sample by quartering, and this quantity should be immediately bottled and then sealed.

Wood in Chips.—As the wood comes from the chipping machine samples should be taken at regular intervals and placed in a large vessel which can be closed so as to prevent loss of moisture. The

total sample is then divided by quartering until a suitable quantity remains. 500 grm., at least, shall be taken for each sample.

Fruits, Roots, Galls, Etc., in Sacks or Bales.—(Myrobalans, divi-divi, algarobilla, valonia, canaigre, galls, teri, gonakie, etc).—The contents of the calculated number of sacks or packages shall be spread upon a clean and level floor so as to form a level and square-shaped layer. The pile is then halved and this process repeated until a suitable quantity has been obtained. 500 grm. are to be taken for each sample.

Valonia.—The tannin content of valonia beard is higher than that of the cups so it is advisable to carry out the analysis on whole cups with the beard intact.

Myrobalans.—The thin and lean nuts are usually lighter than the plumper (richer in tannin) nuts. This should be carefully noted, and samples drawn accordingly.

Sumach.—Adulteration of sumach has been shown to take place in layers, the richest sumach being placed at the top of the bag. Sampling should be carried out by employing an instrument similar to a large cheese or butter sampler, which should be plunged well in to the bags to ensure even sampling.

Liquid Extracts in Barrels.—The calculated number of barrels shall, in the event of these being numbered, be taken from numbers evenly distributed over the total consignment. Before sampling, the contents of the barrels should be thoroughly mixed by rolling. This procedure can be assisted by running out from the barrel two buckets of the extract before rolling. After the sediment has been thoroughly mixed the contents of the two buckets are returned to the barrel and collected into one vessel, well mixed, and samples of 150 grm. taken, bottled and sealed.

If the barrels have been standing for a long period, rolling the barrels is not satisfactory, and the contents of barrels should be well stirred after removing the end.

Undue exposure of the liquid extract to the air should be avoided as much as possible. Extracts that contain frozen material should not be sampled, as the thawing that is necessary by means of steam alters the moisture content.

Liquid Extracts in Tank Waggons.—Two methods may be used:

1. The extract can be run out into a large tank and well "plunged up" until thoroughly mixed. Equal samples shall then be taken

from different parts of the tank, well mixed, and samples of 150 c.c. drawn, bottled and sealed.

2. While the tank waggon is being unloaded five samples of 2 litres each shall be taken at the following times: Three minutes after the extract has commenced to run out, three minutes before the tank is emptied, and the three other samples at equal intervals between the first and last.

Samples of 150 grm. are taken from the well-mixed 10 litres so obtained, bottled and sealed.

Solid Extracts in Bags.—(Quebracho, mangrove, mimosa, chestnut, sumach, valonia etc.). The calculated number of bags shall be selected from as uniformly distributed parts of the consignment as possible. Sub-samples of as nearly equal size as possible shall be taken, representing approximately the relative amounts of inner and outer portions of the extract. The whole of these sub-samples shall then be broken up until they will pass through a sieve of 1 inch mesh and then reduced to the required bulk, by quartering. Samples of 150 grm. at least shall then be drawn, employing a small flat scoop, from opposite corners of the pile. It is advisable, especially in hot weather, to wrap the sample in paraffined paper.

Pasty Extracts (Gambier, Etc.).—The calculated number of bags shall be sampled by means of an instrument similar to a large cork-borer, a hollow copper cylinder ¾ inch in diameter, provided with a copper rod that just fits inside the cylinder so as to remove the core of pasty extract.

The sampler shall be passed completely through each block that is to be sampled in seven places so as to represent inner and outer parts and the portions so obtained mixed together with as little exposure to air as possible. Each sample shall contain 200 grm.

It is sometimes necessary to sample so called solid extracts which are in a pasty condition in a similar manner.

Spent Tanning Materials.—Samples of spent tanning materials shall be taken from the top, middle and bottom and in all these cases from the centre and outer portions of the leaching vessel. These sub-samples shall be thoroughly mixed, reduced in bulk by quartering and samples of 1 kilogram bottled and sealed.

Tan Liquors.—The liquor shall be well mixed by plunging and 1500 c.c. must be taken for each sample. Precautions must also be taken to prevent fermentation setting in.

Preservation of Samples from Moulds and Other Fermenting Agencies.

1. Pasty Extracts.—A few drops of turpentine are shaken in the sample bottle before introducing the sample.

2. Liquid Extracts.—0.5 c.c. of the following reagent shall be added to each 1000 grm. of extract before bottling:

10 gr. HgI_2 and 10 grm. KI in 100 c.c. water.

3. Tan Liquors.—0.03% of thymol shall be added to each litre of tan liquor in the sample.

The above methods are the official methods of sampling of the Society of Leather Trades' Chemists, and samples taken by methods other than those specified will not be recognised as official.

THE "INTERNATIONAL" METHOD OF TANNING ANALYSIS

This is the Official Method for Germany, Holland, Sweden and Norway

General Regulations.—The executive committee have decided that any method which conforms to the conditions of sections 1 to 4 of the following statement may be regarded as conforming to the recommendations of the International Commission on Tannin Analysis, but that members of the International Association must work according to the detailed directions contained in Sections 5 to 6.

1. *The Solutions for Analysis.*—This must contain between 3.5 and 4.5 grm. of tanning matter per litre, and solid materials must be extracted, so that the greater part of the tannin is removed at a temperature not exceeding 50°.

2. *Total Solubles.*—These must be estimated by the evaporation of a measured quantity of the solution previously filtered till optically clear both by reflected and transmitted light; that is, a bright object such as an electric light filament must be distinctly visible through at least 5 cm. thickness, and a layer of 1 cm. deep in a beaker placed in a good light on black glass or black glazed paper must appear dark and free from opalescence when viewed from above. Any necessary mode of filtration may be employed, but if such filtration causes any appreciable loss when applied to a clear solution, a correction must be estimated and applied as described in Section 6. Filtration must take place at a temperature between 15° and 20°, or the actual temperature shall be stated on the Report.

3. *Total Solids.*—These must be estimated by drying a weighed portion of the material, or a measured portion of its uniform turbid solution at a temperature not exceeding 100° *in vacuo* or 105° in air till constant. "Moisture" is the difference between 100 and the percentage of total solids, and "Insolubles" the difference between "Total Solids" and "Total Solubles."

4. *Non-tannins.*—The solution must be detannised by shaking with chromed hide-powder till no turbidity or opalescence can be produced in the clear solution by salted gelatin. The chromed powder must be added in one quantity equal to 6 to 6.5 grm. of dry hide per 100 c.c. of the tanning solution, and must contain not less than 0.5 and not more than 2 per cent. of chromium reckoned on the dry weight, and must be so washed that in a blank experiment with distilled water, not more than 5 mg. of solid residue shall be left on evaporation of 100 c.c. All water contained in the powder should be estimated and allowed for as water of dilution.

I. A. L. T. C. Official Method

The following sections give the detailed method of carrying out the analysis adopted by the I. A. L. T. C. for the use of its own members.

5. *Preparation of Infusion.*—Such a quantity of material shall be employed as will give a solution containing as nearly as possible 4 grm. of tanning matter per litre, and not less than 3.5 nor more than 4.5 grm. Liquid extracts shall be weighed in a basin or beaker and washed with boiling distilled water into a litre flask, filled up to the mark with boiling water, and well mixed, and rapidly cooled to a temperature between 15° and 20°, after which it shall be accurately made up to the mark, again well mixed, and filtration at once proceeded with; sumach and myrobalans extracts should be dissolved at a lower temperature.

Solid extracts shall be dissolved by stirring in a beaker with successive quantities of boiling water, the dissolved portions being poured into a litre flask, and the undissolved being allowed to settle and treated with further portions of boiling water. After the whole of the soluble matter is dissolved the solution is treated similarly to that of a liquid extract.

Solid tanning materials previously ground till they will pass through a sieve of 5 wires per cm. are extracted in Koch's or Procter's extractor with 500 c.c. of water at a temperature not exceeding 50°

and the extraction continued with boiling water till the filtrate amounts to 1 litre. It is desirable to allow the material to soak for some hours before commencing the percolation which should occupy not less than 3 hours, so as to extract the maximum of tannin. Any remaining solubles in the material must be neglected, or reported separately as "difficultly soluble" substances. The volume of liquid in the flask must after cooling be accurately made up to 1 litre.

6. *Filtration.*—The infusion shall be filtered till optically clear (3) (see Sect. 2). No correction for absorption is needed for the Berkefeld candle, or for S. and S. 590 paper if a sufficient quantity (250–300 c.c.) is rejected before measuring the quantity for evaporation; and the solution may be passed through repeatedly to obtain a clear filtrate. If other methods of filtration are employed, the average correction necessary must be estimated in the following manner: About 500 c.c. of the same or a similar tanning solution are filtered perfectly clear, and after thorough mixing 50 c.c. are evaporated to estimate "total soluble No. 1." A further portion is now filtered in the exact method for which the correction is required (time of contact and volume rejected being kept as constant as possible) and 50 c.c. are evaporated to estimate "total soluble No. 2." The difference between No. 1 and No. 2 is the correction sought, which must be added to the weight of the total solubles found in analysis. An alternative method of estimating correction, which is equally accurate and often more convenient, is to filter a portion of the tanning solution through the Berkefeld candle till optically clear, which can generally be accomplished by rejecting 300 or 400 c.c. and returning the remaining filtrate repeatedly; and at the same time to evaporate 50 c.c. of clear filtrate obtained by the method for which correction is required, when the difference between the residues will be the correction sought.

Note.—It is obvious that an average correction must be obtained from at least 5 estimations. It will be found that this is approximately constant for all materials, and amounts in the case of S. and S. 605, 150 c.c. being rejected, to about 5 mg. per 50 c.c., and where 2 grm. of kaolin are employed in addition, to 7½ mg. The kaolin must be previously washed with 75 c.c. of the same liquor, which is allowed to stand 15 minutes and then poured off. Paper 605 has a special absorptive capacity for a yellow colouring matter often contained in sulphited extracts.

It is proposed that the Commission should be asked to estimate average corrections for the more important methods of filtration and report at an early date.

7. *Detannisation.*—The hide-powder employed shall be of a woolly and not granular texture, thoroughly de-limed, preferably with hydrochloric acid, and not requiring more than 5 c.c. of N/10 NaOH or KOH to produce a permanent pink with phenolphthalein on 6½ grm. of the dry powder suspended in water (4); and the detannisation shall be conducted in the following manner:

The moisture in the air-dried powder is estimated, and the quantity equal to 6.5 grm. of actual dry hide-powder is calculated, which will be practically constant if the hide powder be kept in an air-tight vessel. Any multiple of this quantity is taken according to the number of analyses to be made, and made wet again with approximately ten times its weight of distilled water; 2 grm. per hundred of dry powder of crystallised chromic chloride ($Cr_2Cl_6 12H_2O$) are now dissolved in water and made basic with 0.6 grm. of Na_2CO_3 by the gradual addition of 11.25 c.c. of N/I solution, thus making the salt correspond to the formula $Cr_2CL_3(OH)_3$ (5). This solution is added to the powder and the whole churned slowly for 1 hour. In laboratories where analyses are continually being made it is more convenient to use a 10 per cent. stock solution, made by dissolving 100 grm. of $Cr_2Cl_6 12H_2O$ in a little distilled water in a litre flask, and very slowly adding a solution containing 30 grm. of anhydrous sodium carbonate, with constant stirring, finally making up to the mark with distilled water and well mixing. Of this solution, 20 c.c. per 100 grm. or 1.3 c.c. per 6.5 grm. of dry powder should be used.

At the end of one hour the powder is squeezed in linen to free it as far as possible from the residual liquor, and washed and squeezed finally with distilled water, until on adding to 50 c.c. of the filtrate, 1 drop of 10% K_2CrO_4 and 4 drops N/10 $AgNO_3$, a brick-red colour appears. Four or five squeezings are usually sufficient. Such a filtrate cannot contain more than 0.001 grm. of NaCl in 50 c.c.

The powder is then squeezed to contain 70–75 per cent. of water, and the whole weighed. The quantity, Q, containing 6.5 grm. of dry hide is thus found, weighed out and added immediately to 100 c.c. of the unfiltered tannin infusion, together with $(26.5 - Q)$ of distilled water (6). The whole is corked up and agitated for 15 minutes in a rotating bottle at not less than 60 revolutions per

minute (7). It is then squeezed immediately through linen, 1 grm. of kaolin added to the filtrate, stirred and filtered through a folded filter of sufficient size to hold the entire filtrate, which is returned till clear, and 60 c.c. of the filtrate are evaporated and reckoned as 50 c.c., or the residue of 50 c.c. is multiplied by $6/5$. The non-tannin filtrate must give no turbidity with a drop of a 1 per cent. gelatin in 10 per cent. salt solution (8).

OFFICIAL METHOD OF THE AMERICAN LEATHER CHEMISTS' ASSOCIATION FOR THE ANALYSIS OF VEGETABLE MATERIALS CONTAINING TANNIN

I. Raw and Spent Materials

Preparation of Sample.—The sample must be ground to such a degree of fineness that the entire sample will pass through a sieve of 20 meshes to the inch (linear).

(*a*) The temperature used for drying samples of spent material for grinding must not exceed 60°.

(*b*) Samples of raw material too wet to be ground may be dried before grinding as in (*a*). In this case a preliminary water estimation must be made according to (IV) on the sample as received. If the portion of the sample taken for the water estimation is in pieces too large to dry properly, it is permissible to reduce these to smaller size as rapidly and with as little loss of water as possible.

Water Estimation.—Ten grm. of the ground material shall be dried in the manner and for the period specified for evaporation and drying in extract analysis (see IV).

Amount of Sample to be Extracted.—Such an amount of raw material shall be extracted as will give a solution containing as nearly as practicable 0.4 grm. tannin to 100 c.c. (not less than 0.375 or more than 0.425). Of spent materials, such an amount shall be taken as will give a solution of as nearly as practicable the above concentration.

Extraction.—Extraction shall be conducted in an apparatus consisting of a vessel in which water may be boiled and a container for the material to be extracted. The container shall be provided above with a condensation chamber so arranged that the water formed from the condensed steam will drip on the material to be extracted, and provided below with an arrangement of outlets such

that the percolate may either be removed from the apparatus or be delivered to the boiling vessel. The boiling vessel must be so connected that it will deliver steam to the condensation chamber and that it may receive the percolate from the container. The condensation water from the condenser must be at approximately the boiling temperature when it comes in contact with the material to be extracted.

The material of which the boiling flask is composed must be inert to the extractive solution. Suitable provisions must be made for preventing any of the solid particles of the material from passing into the percolate.

(*A*) *Woods, Barks and Spent Materials.*—Five hundred c.c. of the percolate shall be collected outside in approximately 2 hours and the extractions continued with 500 c.c. for 14 hours longer by the process of continuous extraction with reflux condenser. The applied heat shall be such as to give condensation approximating 500 c.c. in 1½ hours.

(*B*) *Materials Other than Woods, Bark and Spent.*—Digest the material in the extractor for 1 hour with water at room temperature, and then extract by collecting 2 litres of percolate outside in approximately 7 hours.

Analysis.—The percolate shall be heated to 80°, cooled, made to the mark and analysed according to the official method for extracts.

II. Analysis of Extract

Amount and Dilution for Analysis.—(*A*) *Fluid Extracts.*—Fluid extracts shall be allowed to come to room temperature, be thoroughly mixed, and such quantity weighed for analysis as will give a solution containing as nearly as possible 0.4 grm. tannin to 100 c.c. (not less than 0.375 nor more than 0.425). Precautions must be taken to prevent loss of moisture during weighing. Dissolve the extract by washing it into a litre flask with 900 c.c. of distilled water at 85°.

Cooling.—(*a*) The solutions prepared as above shall be cooled rapidly to 20° with water at a temperature of not less than 19°, be made to the mark with water at 20°, and the analysis proceeded with at once, or

(*b*) The solution shall be allowed to stand over-night, the temperature of the solution not being permitted to go below 20°, be brought

to 20° with water at not less than 19°, be made to the mark with water at 20° and the analysis proceeded with.

(*B*) *Solid and Powdered Extracts.*—Such an amount of solid or powdered extract as will give a solution of the strength called for under liquid extracts shall be weighed in a beaker with proper precautions to prevent change of moisture. One hundred c.c. of distilled water at 85° shall be added to the extract and the mixture placed on the water-bath, heated and stirred until a homogeneous solution is obtained. When dissolved, the solution shall immediately be washed into a litre flask with 800 c.c. of distilled water at 85°, be cooled, etc., as under (*A*) above.

Note.—It is permissible to make up 2-litre instead of 1-litre solutions, dissolving by washing into flask with 1800 c.c water at 85° in case of fluid extracts, and 1700 c.c. water at 85° in case of solid or powdered extracts.

Total Solids.—Thoroughly mix the solutions; pipette 100 c.c. into tared dish, evaporate and dry as directed under "Evaporation and Drying." (See IV.)

Water.—The water content is shown by the difference between 100% and the total solids.

Soluble Solids.—S. & S. No. 590 or Munktell's No. 1F, 15 cm. single, pleated, filter paper shall be used for the filtration.

The kaolin used shall answer the following test: 2 grm. kaolin digested with 200 c.c. of distilled water at 20° for 1 hour shall not give more than 1 mg. of soluble solids per 100 c.c., and shall be neutral to phenolphthalein. To 1 grm. kaolin in a beaker add sufficient solution to fill the paper, stir and pour on paper. Return filtrate to paper when approximately 25 c.c. have collected, repeating operation for 1 hour, being careful to transfer all kaolin to the paper. At the end of the hour remove solution from filter paper, disturbing the kaolin as little as possible. Bring so much as needed of the original solution to exactly 20°, as described above, refill the paper with this solution, and begin to collect the filtrate for evaporating and drying so soon as it comes clear. The paper must be kept full and the temperature of the solution on the filter must not fall below 20° nor rise above 20° during this part of the filtration. The temperature of the solution used for refilling the paper must be kept uniformly at 20°, and the funnels and receiving vessels must be kept covered.

Pipette 100 c.c. of clear filtrate into tared dish; evaporate and dry as under IV.

Insolubles.—The insoluble content is shown by the difference between the total solids and the soluble solids, and represents the matters insoluble in a solution of the concentration used under the temperature conditions prescribed.

Non-tannins.—The hide powder used for the non-tannin estimation shall be of woolly texture, well de-limed, and shall require between 12 and 13 c.c. of N/10 NaOH to neutralise 10 grm. of the absolutely dry powder.

(*a*) Digest the hide powder with 10 times its weight of distilled water till thoroughly soaked. Add 3 per cent. of chrome alum, $(Cr_2SO_4)_3K_2SO_4.24H_2O$, in 3 per cent. solution calculated on the weight of the air-dry powder. Agitate frequently for several hours and leave over-night. Squeeze and wash by digesting with 4 successive portions of distilled water, each portion equal in amount to 15 times the weight of the air-dry powder taken. Each digestion shall last for 15 minutes, and the hide powder shall be squeezed to approximately 75% of water after each digestion except the last, a press being used if necessary. The wet hide powder used for the analysis shall contain as nearly as possible 73% of water (not less than 71% nor more than 74%). Estimate the moisture in the wet hide powder by drying approximately 20 grm. (See IV). To such quantity of the wet hide as represents as closely as practicable $12\frac{1}{2}$ grm. (not less than 12.2. nor more than 12.8) of absolutely dry hide add 200 c.c. of the original analysis solution and shake immediately for ten minutes in some form of mechanical shaker. Squeeze immediately through linen, add 2 grm. of kaolin (answering test described under *Water*) to the detannised solution and filter through single folded filter (No. 1F Swedish recommended) of size sufficient to hold the entire filtrate, returning until clear. Pipette 100 c.c. of filtrate into tared dish, evaporate and dry as under IV.

The weight of the non-tannin residue must be corrected for the dilution caused by the water contained in the wet hide powder.

Funnels and receiving vessels must be kept covered during filtration. Flasks graduated to deliver 200 c.c. are recommended for measuring the analysis solution to be detannised.

(*b*) Digest the hide powder with the amount of water and add the amount of chrome alum in solution directed under (*a*).

Agitate in some form of mechanical shaker for 1 hour, and proceed immediately with washing and subsequent operations as directed under (*a*).

Note.—In order to limit the amount of dried hide powder used, estimate the moisture in the air-dry powder and calculate the quantity equal to 12½ grm. of actual dry hide powder. Take any multiple of this quantity according to the number of analyses to be made, and after chroming and washing as directed, squeeze to a weight representing as nearly as possible 73 per cent. of water. Weigh the whole amount, and divide by the multiple of the 12½ grm. of actual dry hide powder taken to obtain the weight of wet hide powder for 200 c.c. of solution.

Tannin.—The tannin content is shown by the difference between the soluble solids and the corrected non-tannins, and represents the matters absorbable by hide under the conditions of the prescribed methods.

III. Analysis of Liquor

Dilution.—Liquors shall be diluted for analysis with water at room temperature so as to give as nearly as possible 0.7 grm. of solids per 100 c.c. of solution. Should a liquor be of such character as not to give a proper solution with water of room temperature it is permissible to dilute with water at 80° and cool rapidly as described under (*Amount and Dilution for Analysis, A, a*).

Total Solids.—To be estimated as in Extract Analysis.

Soluble Solids.—To be estimated as in Extract Analysis.

Insolubles.—Estimated as in Extract Analysis.

Non-tannins.—To be estimated by shaking 200 c.c. of solution with an amount of wet chromed hide powder, containing as nearly as possible 73 per cent. water, corresponding to an amount of dry hide powder shown in the following table:

Tannin range per 100 c.c.	Dry powder per 200 c.c.
0.35 – 0.45 grm.	9.0 – 11.0 grm.
0.25 – 0.35 grm.	6.5 – 9.0 grm.
0.15 – 0.25 grm.	4.0 – 6.5 grm.
0.00 – 0.15 grm.	0.0 – 4.0 grm.

Solutions to be shaken for non-tannins as in Extract Analysis and 100 c.c. evaporated as in Extract Analysis.

IV. Temperature, Evaporation and Drying, Dishes

Temperature.—The temperature of the several portions of each solution pipetted for evaporation and drying, that is, the total solids, soluble solids and non-tannins must be identical at the time of pipetting.

Evaporation.—All evaporation and drying shall be conducted in the form of apparatus known as the "Combined Evaporator and Dryer" at a temperature not less than 98°. The time for evaporation and drying shall be 16 hours.

Dishes.—The dishes used for evaporation and drying of all residues shall be flat-bottomed glass dishes of not less than 2¾ inches diameter nor more than 3 inches in diameter.

V. Estimation of Total Acidity of Liquors

Reagents.—(*a*) One per cent. solution of gelatin neutral to hæmatin. The addition of 25 c.c. of 95% alcohol per litre is recommended to prevent frothing. If the gelatin solution is alkaline, neutralise with N/10 acetic acid, and if acid, neutralise with N/10 sodium hydroxide.

(*b*) Hæmatin. A solution made by digesting hæmatin in cold neutral 95% alcohol in the proportion of ½ grm. of the former to 100 c.c. of the latter.

(*c*) Acid-washed kaolin free from soluble matters.

(*d*) N/10 sodium hydroxide.

Directions.—To 25 c.c. of liquor in a cylinder that can be stoppered, add 50 c.c. of gelatin solution, dilute with water to 250 c.c., add 15 grm. of kaolin, and shake vigorously. Allow the mixture to settle for at least 15 minutes, remove 30 c.c. of the supernatant solution, dilute with 50 c.c. of water, and titrate with N/10 sodium hydroxide, using hæmatin solution as the indicator. Each c.c. of N/10 sodium hydroxide is equivalent to 0.2% of acid, as acetic acid.

VI. General

When materials containing sulphite-cellulose extract are analysed, the fact that the material contains sulphite-cellulose extract shall be noted on the report.

The test for the presence of sulphite-cellulose in a liquor or extract shall be as follows: 5 c.c. of a solution of analytical strength shall be placed in a test-tube, 0.5 c.c. of aniline added, and the whole well

shaken; then 2 c.c. of strong hydrochloric acid are added and the mixture again shaken. If at least as much precipitate remains as is obtained when a comparison solution prepared as below is similarly treated, the material shall be held to contain sulphite-cellulose.

The comparison solution shall consist of sulphite-cellulose in the proportion of one part total solids to 2000 c.c. of solution, and as much tanning material, similar to that being tested, but known to be free from sulphite-cellulose, as will make up the solution to analytical strength. Attention is drawn to the fact that certain synthetic tannins give precipitates under the conditions of this method.

On public analytical work by members of this Association the fact that the Official Method has been used, shall be so stated.

Official Method for Sampling Tanning Materials

General.—Extracts whether liquid or solid, and tanning materials in general all contain moisture. The amount of moisture varies with climatic conditions, but especially in liquid, and in most solid extracts becomes less as the extract is exposed to the air. As the value of any material shown by analysis is directly dependent upon the amount of moisture contained, and as an exposure of a comparatively few moments may alter appreciably the amount of moisture, it is apparent that the sampling in all its details should be done as quickly as is consistent with thoroughness and with great care to expose the material as little as possible to the air. The portions taken as samples should be placed at once in containers as nearly air-tight as possible, and preferably of glass. Wood, cardboard, poorly glazed crockery, etc., are all porous and more or less absorbent and not suitable for retaining samples.

Liquid extract cannot be accurately sampled when it contains any frozen material. A sample of extract taken after live steam has been run into the extract has not the same concentration as the original extract. A sample of spent bark which has been standing where dust from fresh ground bark has sifted into it does not represent the degree of extraction of the spent bark. Samples of liquor which have been kept with no preservative in them for some time do not represent the condition of the liquor when sampled.

All extracts and crude tanning materials shall be sampled as nearly as possible at time of weighing, and for every 50,000 pounds, or less, sampled a sample shall be drawn.

Solid, Powdered and Pasty Extracts.—The number of packages to be sampled out of a given lot shall be ascertained by taking a percentage of the total number of packages in the lot obtained in the following manner: Divide the total number of packages by 100, multiply by 0.02 and subtract from 4. Thus

$$4700 \div 100 = 47$$
$$47 \times 0.02 = 0.94$$
$$4 - 0.94 = 3.06 \text{ per cent.}$$
$$4700 \times 0.0306 = 144 \text{ packages.}$$

For lots of 200 packages and under, 5% of the number of packages shall be sampled, and for lots of 10,000 packages, and over, 2% of the number of packages shall be sampled.

Whenever possible, every Nth. package shall be set aside for sampling while the extract is being moved. When this is not possible, the packages shall be selected from as uniformly distributed parts of the bulk as possible.

Samples of as nearly equal size as practicable shall be taken from each package and these samples shall represent as nearly as may be, proportionally the outer and inner portions of the extract. These sub-samples shall be placed in a clean, dry, closed container. When sampling is completed, the whole composite sample shall be broken up until it will pass through a sieve of 1-inch mesh; it shall be reduced to the required bulk by successive mixings and quarterings. From this bulk duplicate samples of at least 6 ounces shall be drawn from opposite quarters by means of a small flat scoop (and not by selecting a handful here and there). The sample shall be enclosed in the smallest clean, dry, glass receptacle, sealed and properly labelled.

Note.—Whenever possible, the sample should be wrapped in paraffin paper and placed in the smallest straight-side glass receptacle; especially is this desirable during the warmer months of the year.

Sampling at place of manufacture shall be conducted by running a portion from the middle of each strike into a mould holding at least 2 pounds. These sub-samples shall be preserved with proper precautions against evaporation, and be sampled for analysis as above.

Liquid Extracts in Barrels.—The number of barrels of extracts to be sampled out of any given lot shall be not less than 10 per cent. of

the whole number of barrels for every 50,000 pounds or fraction thereof. The barrels to be sampled shall be rolled and shaken from end to end until the contents are homogeneous. Whenever this is not possible the heads of the barrels shall be removed and the contents stirred until homogeneous, a sample of equal size to be taken from each barrel. These sub-samples shall be put together in a suitable closed container and be thoroughly mixed. From this bulk duplicate samples of at least 4 ounces shall be drawn and preserved in clean, dry, glass containers; sealed and labelled with such distinguishing marks as may be necessary.

Liquid Extract in Bulk.—The extract shall be agitated with air, be plunged or be mixed by some other efficient means until homogeneous. Equal samples shall then be taken from different parts of the bulk, be placed in a proper container, be thoroughly mixed and sampled as described in *Liquid Extracts in Barrels.*

Liquid Extract in Tank Cars.—The following methods are permissible:

(*a*) The extract shall be unloaded into clean, dry containers and sampled according to *Liquid Extract in Bulk;* or

(*b*) The extract shall be mixed until homogeneous, by plunging through the dome or by other effective means, then numerous equal samples shall be taken from as widely scattered parts of the bulk as possible. These samples shall then be placed in a suitable container, be mixed and sampled as in *Liquid Extracts in Barrels.*

Note.—As it is almost impossible to secure a homogeneous mixture of the extract in a tank car, this method should be used only when no other is possible. Or,

(*c*) The extract shall be sampled as follows while the car is being unloaded. A quart sample shall be taken from the discharge three minutes after the extract has begun to run; another quart sample shall be taken three minutes before the extract has all run out, and three other quart samples shall be taken at equal intervals between these two. These five samples shall be transferred to a suitable container as soon as taken, be thoroughly mixed and sampled as in *Liquid Extracts in Barrels.*

Crude Tanning Materials.—(*A*) Shipments in bags, mats or other similar packages.

A number of packages shall be sampled representing 2% of the weight for every shipment of 50,000 pounds or fraction thereof,

by taking representative portions from each package. These sub-samples shall be mixed together and the bulk be reduced by mixing and quartering to the desired size. Duplicate samples of not less than 5 pounds each shall be preserved in air-tight containers properly labelled.

(*B*) Shipments in bulk, bark, wood, etc., in sticks.

Sticks shall be taken from at least ten uniformly distributed parts of the bulk, be sawed completely through and the sawdust thoroughly mixed and sampled as in "(*A*)."

(*C*) Materials prepared for leaching.

Samples of equal size shall be taken at uniform intervals as the material enters the leach and be kept in a suitable container till sampling is completed. This bulk shall then be thoroughly mixed, be reduced by mixing and quartering, and duplicate samples for analyses of at least 2 pounds in size be preserved in air-tight containers, as in "(*A*)."

Spent Materials from Leaches.—Samples of spent material shall be taken from the top, middle and bottom, and in each case from the centre and outer portions of the leach. These sub-samples shall be thoroughly mixed, be reduced in bulk by mixing and quartering, and duplicate samples of at least 1 quart in size be preserved for analysis.

Tanning Liquors.—The liquor shall be mixed by plunging or other effective means till homogeneous and then samples of at least 1 pint be taken for analysis. The addition of 0.03% of thymol or other suitable anti-ferment to the sample is essential to keep the liquor from altering its original condition.

When routine samples are taken from day to day and a composite sample analysed, samples of equal size shall be taken from each vat after thorough mixing, be preserved in covered containers in as cool a place as possible, and be kept from fermentation by the addition of suitable anti-ferment, as above. This bulk shall be mixed till homogeneous and samples of not less than 1 pint each be preserved for analysis.

When a sample is taken by a member of this Association in accordance with the above method, it is requested that he state upon the label of the sample submitted and upon the analysis blank that "this sample has been taken in accordance with the official method of sampling of The American Leather Chemists' Association."

TANNIN ANALYSIS OF LIQUORS AND SPENT TANNINS

Total Solids.—This is only important if it is desired indirectly to estimate suspended matter. The liquor is well shaken and 50 c.c., or less, is measured into a basin, evaporated to dryness and dried till constant at 105 to 110°.

In most cases the liquor may at once be filtered, either through a Berkefeld candle, or through paper, kaolin being used if necessary to get it perfectly clear. The specific gravity should always be taken with a pyknometer or hydrometer. Barkometer degrees correspond to the second and third decimals of specific gravity; thus sp. gr. 1.065 = 65 Bkr.

Total Dissolved Matter.—50 c.c, or a smaller quantity of strong liquor, is evaporated to dryness in a weighed basin, dried to constant wt. at 100 to 105°, and weighed. In stronger liquors, 5 or 10 c.c. is quite sufficient. Difficulty is likely to arise from the presence of partially volatile substances and especially of lactic acid.

Suspended matter is estimated by subtracting the "dissolved matter" from the "total solids." The calculation is only reliable in dilute solutions, where the *volume* of the suspended matter may be neglected. In other cases the suspended matter may be collected on a tared filter, weighed wet with the filter and again when dry, and the weight of dissolved matter calculated corresponding to the loss of water and deducted from the net weight.

Ash and Organic Matter.—The residue of the "total soluble" (or "total solids") is cautiously incinerated, moistened with ammonium carbonate solution, evaporated to dryness, and cautiously heated to incipient redness to drive off ammonium salts without decomposing carbonates. The loss is total "organic matter," the residue "mineral constituents," which consist mainly of lime, and may be further analysed. The ash may be dissolved in excess of N/1 HCl, and titrated back, when bases found will have existed in the liquors as salts or organic acids. These neutral salts have very considerable influence on the tanning process, and appear to be one of the principal causes of the "mellow" action of old liquors. The estimation of iron in the ash is frequently of great importance, and may be made colorimetrically.

Tanning Matter.—A portion of the filtered liquor is diluted or concentrated, till it contains about 4 grm. of tanning matter per

litre, and is analysed as described in Section XIII by the chrome-chloride method. Where very large quantities of non-tans are present, it is often necessary to reduce the tannins below the official strength of 4 grm. per litre. The residues of "total soluble" and "non-tannins" may be redissolved in water, and the acid in each estimated (see below), and the difference, reckoned as lactic or gallic acid, as the case may be, deducted from the tanning matter found. Where the proportion of tannin is small, great accuracy is impossible from the disturbing action of non-tannins.

The results of analysis of liquors may be stated in grm., either per 100 grm. (%), or per 100 c.c. (volume %); but it must be made clear which is used, and the density of the liquor must always be given to render it possible to calculate from one to the other.

The Löwenthal method may also be used for tannin estimation, especially for weak and acid liquors, or where a series of estimations are required. The tannin may be reckoned in terms of gallotannin, when it will be always lower than the "tanning matter" estimated by hide-powder; or a factor may be obtained by simultaneous hide-powder estimations of one or more of the liquors.

Valuation of Liquors.—The chemist is often called upon to make an estimate of the value of liquors in a tannery, but so many circumstances are involved that at most a somewhat rough approximation can be given. It is generally out of the question to analyse each liquor individually, and it is sufficient to estimate the amount of "tanning matter" per barkometer degree in a few different stages of the process, and calculate the value of the other liquors from their barkometer strength. It will be found that the tannin strength diminishes much more rapidly than the barkometer degree as the liquors become weaker, because only the tannin matter is absorbed by the skin, while the non-tannins accumulate. The proportion of the two may be approximately estimated from the hide-powder analysis. The different tanning value of liquors in different parts of the yard will be best adjusted by deducting a fixed number of barkometer degrees in each case before estimating value. Thus, if a layer of liquor of 50 barkometer contains 4% of tanning matter, and a handler liquor of 20 only 1%, we may deduct 10 throughout and say that each degree above 10 is equal to 0.1% of tannin. If the analysis and value of the materials are known, it is easy to estimate the cost value of 1% of tanning matter; remembering that carriage,

and with solid materials cost of grinding, must be added to the original price, and that the tanning value of materials shown by analysis must be diminished by an allowance for loss in leaching.

If the actual cost or value of the materials is not known, average strengths and market values must be taken. The weight of the liquors may be conveniently taken at 10 lb. per gallon, or 62.25 lb. per cb. ft. and this will be strictly accurate if the liquors are measured instead of weighed for analysis.

Pits containing hides and dusting materials do not usually contain more liquor than two-thirds of their total capacity. Value of dust may generally be included with that of the liquor. For stock-taking purposes the value of all materials in the leaches may be taken at half cost, but this is generally an over-estimate.

Spent tanning materials must generally be dried and ground and extracted like other solid materials, somewhat large quantities being used, and the weak liquors concentrated by boiling. For a series of estimations it is often convenient to use the Löwenthal method, which gives good comparative results. In this case concentration is unnecessary.

It is generally most convenient to calculate the percentage on the dry substance, but in comparing the results with the original material, the loss of weight in leaching and drying must not be overlooked. If the composition of the unleached material is known, the original weight of the leached sample may be calculated from the insoluble present. Thus, if the original material contained 45% of insoluble matter, and the spent material 83% the tannin found in the latter would be multiplied by $^{45}/_{83}$ rds, to reduce it to that of an equal original weight. This correction is of the greater importance the stronger the material. Where the actual analysis of the original material is unknown, the corrections must be calculated from that of an average sample.

GENERAL OBSERVATIONS ON HIDE POWDER

Maschke (*Dingler's J.*, 1896, **302**, 46) has examined a number of hide powders, and has shown that a good hide powder contains very little soluble matter. According to his observations a good hide powder gives 0.039 grm. per 50 c.c. water-soluble matter. Weiss (*Der Gerber*, 1905, 260, 276) has carried out similar experiments with "chromated" hide powder. He fixed the maximum

soluble matter to 4–8 mg. per 100 c.c. Bennet (*Collegium*, 1907, 149) has shown that the absorption power of a good hide powder depends on its low acidity. He stipulates that 6.5 grm. of hide powder on extraction with 100 c.c. of water should not require more than 5 c.c. N/10 KOH. This was also confirmed by Small (*J. Amer. Leather Chem. Assoc.*, 1907, **2**, 347).

Jedlicka and Dlonhy (*Collegium*, 1911, 253) have shown that a rise of temperature affects the absorptive power of hide powder. According to their observations a temperature of 28–30° has the most detrimental effect.

Differences in the amount of non-tans obtained have been observed to be due to variations in the hide powder in different samples which actually conform to the official regulations. These absorb varying amounts of non-tans. Stiasny (*Leather Trade Rev.*, 1912, 901). has suggested, to meet this difficulty, that "if two hide powders gave no soluble matters on washing, and if the non-tans solution showed no reaction for tannin, the hide powder giving the higher non-tans should be regarded as correct." It must be remembered, however, that if the error is due to the relative extraction of some substance from the hide powder under the conditions existing during the extraction of the tannin and not to mere water washing, the reverse might be the more correct procedure. The latter condition may quite possibly be responsible for the difference.

Official Methods for the Examination of Hide Powder.—The following has been recommended by the Hide Powder Committee (*Collegium*, London, 1916, 155).

Estimation of Moisture

Official Method.—A stoppered weighing bottle 1¼ inches in diameter is heated for 15 minutes at 100° in the steam oven, with the stopper out, cooled in a desiccator for 20 minutes, with the stopper in, and then weighed; (W_1).

After allowing it to stand for 5 minutes in the balance case, it is reweighed; (W_2). It is then immediately filled with hide powder, about 2 grams being added, the packing being as loose as possible. The stopper is at once replaced and the bottle and contents weighed; (W_3).

The bottle and contents, with the stopper out, are then dried for 3 hours at 100°, preferably in a steam-oven. The stopper is then

replaced, and the bottle and contents cooled in a desiccator for 30 minutes and again weighed. The drying is continued for another hour, to see if the weight is constant, and if not, continued still longer until a constant weight is obtained; (W_4).

The percentage of moisture in the powder will then of course be

$$100\,\frac{(W_3 - W_2) - (W_4 - W_1)}{W_3 - W_2}.$$

A duplicate experiment should be done also, taking the hide powder from a different part of the bulk sample. This result should be reported separately.

Each member of the Committee reported his results by this method to the Secretary, and the average was taken of the mean results of each worker. This Committee average is taken as the official result for moisture in the hide powder, and on this basis the amount of hide powder is calculated equivalent to 6.5 grm. dry hide powder which quantity, rounded to the nearest dcgrm., is accepted as the amount of hide powder to be used in all subsequent work.

The adoption of this standard method has resulted in considerable improvement in the concordance of results by the various members of the Committee. This improvement is best illustrated by the following figures, which show the differences between the highest and lowest percentages of moisture reported on each of the American hide powders tested.

Hide powder	Greatest difference in results
2123	2.5
2124	2.4
B_1	2.3
B_2	1.8
B_3	0.6

Acidity, Soluble Matter and Blank Test.
Official Method

The quantity (R) of the air-dry hide powder which, according to the Committee's average, corresponds to 6.5 grm. dry hide powder is digested for about 10 minutes in an ordinary shake bottle with 100 c.c. of distilled water, the mixture being shaken occasionally by hand. After the addition of 1 c.c. of a 1% alcoholic solution of phenolphthalein, the mixture is titrated with N/10 NaOH until a permanent pink colour is obtained. Results are stated in c.c. N/10 NaOH required.

A duplicate experiment should be made also, the hide powder being taken from a different part of the bulk sample. This result should be reported separately.

The average is taken of the mean results obtained by each member of the Committee, and this average constitutes the official result of the Committee's test. On the basis of this average any necessary correction of acidity is made.

The I. A. L. T. C. regulations stipulate that the acidity of the hide powder before chroming shall be between 2.5 and 5.0 c.c. N/10 NaOH per unit. Any hide powders whose acidity lies outside these limits must be brought within them by the addition of the requisite amounts of N/10 NaOH or N/10 HCl. The Hide Powder Committee, whilst of course loyally adhering to these rules have adopted the principle of minimum interference, viz., that sufficient only of these reagents should be used to bring the hide powder *just* within the regulations. Thus, if a hide powder have an acidity of 5.8, its acidity is brought down to 5.0 *only* by adding 0.8 c.c. N/10 NaOH per unit; and if a hide powder have an acidity of 2.0 it is to be brought up to 2.5 *only*, by the addition of 0.5 c.c. N/10 NaOH per unit before chroming. If a hide powder have an acidity between 2.5 and 5.0 per unit, no additions of either hydrochloric acid or sodium hydroxide must be made. In the case of recent official hide powders no such corrections have been necessary.

Soluble Matter.—Official Method.—The quantity (R) of hide powder, used for each analysis, is placed in an ordinary shake bottle with ($126.5—R$) c.c. of distilled water, and churned slowly for one hour. It is then filtered through linen, and through filter paper, with the assistance of about 1 grm. of kaolin, and 60 c.c. filtrate are evaporated to dryness and dried to constant weight. The weight of the residue multiplied by two gives the "soluble matter in 6.5 grm. dry hide powder." A duplicate experiment is in this case unnecessary.

Blank Test.—Official Method.—This should be carried out in exactly the same way as in an official detannisation, but 100 c.c. of distilled water are employed instead of 100 c.c. tan infusion. Two tests should be carried out, side by side, like duplicate analyses, *i. e.*, the hide powder should be chromed, washed and squeezed in one whole, but separated for the two shakes: 60 c.c. are evaporated in each case, and the residues added together and reported as one result—"Residue of blank test per 6.5 grm. dry hide powder."

A certain proportion of the residue of a blank test will undoubtedly be nitrogenous, and some of this may be precipitated by the tannin in

an ordinary detannisation. Hence the error indicated by a blank test is probably less than is apparent.

Comparative Non-tannin Estimations.—The quantity (R) of hide powder necessary for each analysis having been estimated as above (Part I), and also any correction necessary for acidity (Part II), comparative estimations of soluble non-tannin matters are carried out by the official method of analysis. The proposed new hide powder is tested alongside the hide powder which is then the official hide powder, in order to see how the non-tannin results will vary with the change in hide powder. Usually each member has tested materials in which he has chiefly been interested, the difference between the two hide powders with any one worker, being more important than the concordance possible between different workers on one extract.

These tests have a certain commercial importance, as some dislike a change of hide powder during the execution of a contract. With the last three official powders, however, there could be little objection to this, as all workers obtained much the same results with new and old powders.

The chief importance of this test is that it is a safeguard against the introduction of hide powders which have the property of absorbing large quantities of soluble non-tannin matters in addition to the tannin, thus giving erroneously high figures in the tannin results, and a fictitious value to materials thus tested.

Observations of the Hide Powder Methods.—Since the introduction of hide powder as an adsorbent for tanning, first by Bell-Stephens (1826), and later by Weiss (1887), it has been the object of much work and research. In spite of all this, the hide-powder methods are still open to much criticism. This is particularly evident from the following extract from the Annual Report of the Progress of Applied Chemistry 1918, 332. It summarises the results obtained by a Special Committee. "Sixteen chemists analysed four samples, with disappointing results. The greatest difference between analyses of a powdered chestnut extract was 2.64% of tannin, but in the case of divi-divi, this difference was no less than 12.28%. No doubt there is difficulty in getting uniform samples from a consignment of this material, but for one analyst to find 42.09% of tannin and another 54.37% is disconcerting."

Other Methods of Estimating Tannins.—The direct weighing of the precipitate produced by gelatin in a solution of tannin was first suggested by Sir. H. Davy, in 1804, who stated that the precipitate contained 40% of gallotannin. The method has been more recently employed by Stoddart, Macagno, Günther, Johansen, Lehmann, and others, who differ widely in their statements as to the composition of the precipitate. It undoubtedly varies greatly in composition according to the strength of the solution and other circumstances, besides which it is soluble in excess of gelatin solution and very difficult to wash free from alum or other salts employed to facilitate the precipitation. The variable nature of the precipitate, to say nothing of the difficulty of ascertaining the end of the action, is against the use of this method. Lehmann has shown that the liquid may be diluted within certain limits without notably affecting the result, whilst the clarification of the liquid can be effected by adding powdered glass or barium sulphate and vigorously stirring. The tannin infusion is diluted with an equal volume of saturated aqueous solution of ammonium chloride, and titrated with a 1% solution of gelatin in cold saturated ammonium chloride. The end of the action is ascertained by filtering a few drops of the liquid and testing it with a solution of gelatin on a watch-glass placed on a black surface. *Catechu tannin* is said to give good results in this way, 1 c.c. of the gelatin reagent representing 0.139 grm. of the tannin. Johansen recommends that a little chrome alum should be added to the ammonium chloride solution.

H. Dieudonne (*Chem.-Zeit.*, 1886, **10** 1067) ascertained the density of the infusion before and after the absorption by means of a delicate hydrometer, instead of weighing the residues obtained on evaporating equal volumes to dryness, and gave a table of densities of solutions of gallotannin. The saving of time effected by ascertaining the density of the infusions, instead of evaporating them to dryness is more than counterbalanced by the uncertainty that all tannins have the same solution-density as gallotannin. The suggestion is practically a revival of the obsolete process of Hammer. According to this observer, for concentrations below 5%, gallotannin has a solution-density of 0.004. Above that strength the increase is slightly more rapid, a 10% solution having a sp. gr. of 1.0406, and 15% of 1.0614, whilst a 20% solution has a sp. gr. of 1.0824. Hence

each 0.1 grm. of gallotannin present in 100 c.c. of its aqueous solution may be regarded as increasing the sp. gr. by 0.0004.

Under ordinary circumstances, the direct observation of the increase in the weight of the hide, or other gelatinous substance employed, is impracticable, but purified catgut has been suggested by A. Girard for the estimation of the tannin and colouring matter of *wine* (*Rep. Analyt. Chem.*, 1882, **18**, 285).

W. Schmitz-Dumont (*Zeit. öffent. Chem.*, **3**, 209) proposed as a substitute for hide powder formalin-gelatin prepared in the following way: thick filter-paper is saturated with a 10% solution of gelatin and dried. This is then immersed for 24 hours in a 2% solution of formalin, and afterwards dried at 95°. It is then cut into strips and reduced to powder by grinding, and again treated with formalin solution for 24 hours. It is then dried at 100°. In order to free the preparation from trioxymethylene it is digested in hot water until the washings give no formaldehyde reaction with alkaline resorcinol. The powder is then dried on a water-bath and is ready for use. Hide powder treated with formalin has also been tried, but is unsatisfactory in its keeping qualities.

A modified method of estimating tannin by precipitation with gelatin has been described by Collin and Benoist (*Monit. Scient.*, 1888, **31**, 364). They employ an aniline dye in conjunction with gelatin, and operate in the presence of calcium acetate. The end of the operation is indicated by the decolorisation of the liquid, the dye being precipitated with the gelatin. (The use of magenta as an indicator for titration with quinine was previously suggested by Wagner, but was found useless from the fact that it was freely absorbed by the precipitate of tannate of gelatin.)

A solution of tannin is made by dissolving 5 grm. of dry pure gallotannin in water, adding 0.5 c.c. of a 10% solution of mercuric iodide dissolved in its own weight of potassium iodide, and diluting the liquid to 1 litre. A weight of 5 grm. of gelatin is dissolved in 1 litre of hot distilled water, the liquid boiled, and sufficient white of egg added to clarify it. After cooling, 0.5 c.c. of the mercuric iodide solution is added and sufficient sodium hydroxide to render the liquid slightly alkaline. 50 grm. of pure and dry calcium acetate are dissolved in 1 litre of water, and the filtered liquid treated with a few drops of the mercuric iodide solution. This acts as a preservative of the solution.

For the assay of tannin infusions which are not coloured a 1% solution of pure methylene blue is used; for coloured tannins or extracts either a 4% solution of Nicholson's blue BB, or a 1% solution of blue-black NBI.

For the estimation a flask is used, having a capacity of about 60 c.c. and a neck 3 cm. in diameter. 1 c.c. of gelatin solution, 2 drops of blue solution and 5 c.c. of calcium acetate are measured into the flask, which is then filled to the neck with distilled water at a temperature of 75 to 80°, by means of a burette capable of delivering 40 drops to 1 c.c. A little of the standard solution of tannin is added, after which the flask is closed and shaken. A precipitate is formed which rapidly rises to the surface of the liquid, and the addition of the tannin is continued, drop by drop with agitation between each addition, until the solution becomes colourless. The process is then repeated with a solution of the tannin matter to be assayed, which, if acid, should previously be nearly neutralised by the cautious addition of sodium hydroxide.

The method has been tested under various conditions. Alterations in the concentration of the tannin solutions; the presence of other organic substances, such as lactates, butyrates, gallates, and gallic acid; and all the salts that accompany tannin as it occurs in commerce, have little or no influence on the results. When a large proportion of gallic acid is present, a known volume of the standard tannin solution must be added to the solution to be assayed, and the requisite correction made.

Casein has been used by Nierenstein (*Chem. Zeit.*, 1911, **35**, 31) as a precipitant for tannin in the place of gelatin, 100 c.c. of a solution of tannin being shaken for 10 minutes with 6 grm. of casein (free from fat) and then with a further 3 grm. of the same material. After filtering, the absorbed tannin is estimated as in the hide powder process. It is said that dextrose and gallic acid are not absorbed.

A method of assaying *tea*, originating with Allen (*Chem. News*, **29**, 169, 189) was based on the precipitation of the tannin from a hot solution by a standard solution of lead acetate, the end of the action being ascertained by filtering a few drops of the liquid and testing it with ammoniacal ferricyanide. The method was selected partly because the estimation included any gallic acid which might be present, and hence is not suited for the assay of tanning materials without some modification. It has been stated by Villon (*Bull. Soc.*

Chim., 1887, **47**, 97) that gallic acid is not precipitated by lead acetate, but this is not the case. Guyard suggested that by using a solution of acetate of lead containing a considerable quantity of free acetic acid it might be possible to precipitate tannic acids (and colouring matter) while leaving gallic acid in solution, and then, by treating the lead precipitate with dilute sulphuric acid, a solution would be obtained in which the tannin could be estimated by the permanganate method. This process has been investigated and used in the Dreaper method.

R. Jackson (*Chem. News*, 1884, **50**, 179) agitated tannin infusions with lead carbonate, filtered after a few hours, and calculated the tannin from the loss of gravity, assuming a 1% solution of all kinds of tannin to be 1.0038.

Dodge (*J. Amer. Leather Chem. Soc.*, 1907, **2**, 38) precipitates the tannin by means of lead carbonate, estimating the total solids and soluble solids by the official method (A. L. C. A.). Acid solutions dissolve part of the lead carbonate, and this must be allowed for. Results are rather higher than with hide powder.

A. Carpenè (*Gazzetta*, 1875, **5**, 129) recommends, for the estimation of the tannin in *wine*, the use of a solution of ammonio-acetate of zinc containing a large excess of ammonia, which reagent has the property of forming with the œno-tannin a tannate of zinc quite insoluble in water, in ammonia, and in excess of the reagent itself; while it gives no precipitate with alcohol, malic or tartaric acid, tartrates, glycerin, gelatin, albumin, or the iron salts of organic acids. With gallic and succinic acids, dextrose, and salts of aluminium it forms precipitates soluble in excess of the reagent and in ammonia.

On treating the wine with an excess of ammoniacal zinc acetate, a precipitate is formed, consisting of zinc tannate mixed with a small quantity of colouring matter. The wine is heated nearly to boiling to agglomerate the precipitate, which, after cooling, is filtered off and washed with a little boiling water, to remove adherent colouring matter. The precipitate is dissolved in dilute sulphuric acid, and the solution so obtained titrated with standard permanganate and indigo, as indicated on page 112. The results by this method are stated to be accurate when applied to wine, but Kathreiner found that with ordinary tannin matters the figures were very inconstant.

Figures have been given covering the use of this process with chestnut, mimosa, sumach, and quebracho, which seem to be satis-

factory and agree with the official methods within certain close limits.

Ammoniacal acetate of zinc is recommended to precipitate the tannin by Lepetit (*Collegium*, 1910, 375). The excess of zinc is removed by ammonium sulphide. 20 grm. of zinc acetate are dissolved in 80 c.c. of water and 12 c.c. of ammonium acetate solution added. The latter is prepared by neutralising glacial acetic acid with strong ammonia. 8 c.c. of strong ammonia are then added. To precipitate the tannin, 100 c.c. of solution containing 4.65 grm. of tannin per litre are treated with 6 c.c. of the zinc solution. After five minutes the solution is filtered through S. and S. paper 605. The zinc is removed from the clear filtrate, and 4 drops of acetic acid and 1.5 of colourless ammonium sulphide are added to 65 c.c. of the filtrate. This, after filtration, is evaporated to dryness and the residue dried at 102–105° in a vacuum.

In Gerland's process, the tannin is precipitated by a standard solution of tartar emetic (2.611 grm. per litre), in the presence of ammonium chloride, which prevents the coprecipitation of gallic acid. The assay of *sumach* by this method is said to give results which are constantly 2/3 of those given by titration with permanganate. The tendency of the standard solution to change may be obviated by the addition of methylated spirit to the solution. Some tannins (*e. g.*, those of catechin and horse-chestnut) are not precipitated by tartar emetic.

Richards and Palmer (*Silliman's Amer. J. Science*, **3**, 16, 196, 361) substituted acetate for the chloride of ammonium in Gerland's process, and ascertained the point of complete precipitation of the tannin by testing a drop of the clear supernatant liquid on a hot porcelain plate with solution of sodium thiosulphate, which produces an orange precipitate if the antimony is in excess. The standard solution of tartar emetic contained 6.730 grm. of the dried salt per litre; 1 c.c. of this solution is equivalent to 0.01 grm. of tannin.

The Parker-Payne method of analysis (*J. Soc. Chem. Ind.*, 1904, **23**, 648) is based on the estimation of the acidity of the solution before and after the removal of the tannins as calcium salts. 300 c.c. of N/5 solution of calcium hydroxide are added to 200 c.c. of the tannin solution of about the strength used in the official methods, and allowed to stand for 4 hours with occasional shaking. 100 c.c. are filtered and titrated with acid, phenolphthalein being used as an

indicator. The amount of calcium hydroxide used is called the "*total absorption value.*" The tannin is then removed by precipitation with "collin," an indefinite form of hydrolised gelatin, which is very sensitive as a precipitant. The lime absorption in this filtrate is then taken and gives the "acid absorption," the difference between these figures being, the true tannin. It has been pointed out (Dreaper, *Chem. News*, 1904, **90**, 111, and Wood, *J. Soc. Chem. Ind.*, 1904, **23**, 1071) that this precipitation is not a satisfactory one. Procter and Bennett (*J. Soc. Chem. Ind.*, 1906, **25**, 251) also consider it unsatisfactory. Boegh (*Collegium*, 1904, **125**, 301) also shows that while the process seems to give results which compare with the hide-powder process in the case of the pyrogallol tannins, this is not so with the catechol group.

A. Casali (*Chem. Zeit.*, 1884, **8**, 98) estimated tannin by precipitation with a solution of nickel ammonium sulphate. A volume of solution (1 c.c.) which will precipitate 0.01 grm. of gall-tannin is stated to be equivalent to 0.01497 of oak-bark tannin.

F. Becker described a method of estimating tannin by precipitation with a solution containing 5 grm. of methyl violet per litre (*Chem. Zeit.*, 1885, **9**, 46). 50 c.c. of this solution are diluted with 450 c.c. of water at 50°, and a 1% solution of pure gallotannin run slowly in, with continual stirring, until the colouring matter is completely precipitated, a point readily ascertained by filtering a small sample. A similar experiment is then made with an infusion of the tanning material to be tested. The process is said to be well adapted for the assay of *sumach*, and might be found useful in most cases where the tannin is intended to be employed in dyeing or lake formation.

Ostermeyer, improving on a suggestion of Wagner, proposed to estimate tannin by a standard solution of cinchonine coloured with magenta, the end of the action being indicated by the pink tint acquired by the solution (*Chem. News*, 1879, **40**, 181). Gallic acid is not precipitated by cinchonine. Some observers have reported unfavorably on this process, and state that in certain cases the liquid acquires a red tinge long before the tannin is precipitated. The alkaloid solution contained 4.523 grm. of cinchonine sulphate, with 0.5 c.c. sulphuric acid, and 0.1 grm. fuchsin in 1 litre; each c.c. of this solution is said to precipitate 0.01 grm. of tannin.

Such a method for the analysis of tannin in hops and in tea has been devised by Chapman, and by Tatlock and Thompson, respectively (*Analyst*, 1908, **33**, 95, and *ibid*, 1910, **35**, 103). In both cases, as suggested, the tannin is precipitated from aqueous solution by cinchonine or quinine sulphate. The following particulars are given in the case of tea analysis: 1 grm. of tea is boiled in 400 c.c. water under a reflux condenser for 1 hour. After filtering and bringing the temperature to 15.5° add 1 grm. of ordinary basic quinine sulphate dissolved in a mixture of 25 c.c. water and 2.5 c.c. N sulphuric acid. After 15 minutes the precipitated quinine tannate is collected on a tared filter-paper, any precipitate remaining in the beaker being washed into the filter with some of the filtrate, but not with water. After thorough draining, the precipitate is transferred to a weighed basin and dried at 100°. The weight is multiplied by 0.75 to obtain the tannin. This process is claimed to exclude the estimation of colouring matters as tannin. The following figures are given:

	Tannin %	
	Variations	Average
Indian teas	13.32 to 14.98	14.33
Ceylon teas	10.31 to 13.91	12.29
China teas	7.27 to 10.94	9.50

Chapman gives the following details for the estimation of tannin in hops (*J. Inst. Brewing*, 1907, **15**, 649).

Ten grm. of the hops, taken so as to represent as fair a sample as possible, are put into a glass flask marked at 508 c.c., 400 c.c. of boiling distilled water are then added to the flask, and the hops macerated with the aid of a stout glass rod suitably bent at the end. The flask with its contents is immersed in a water bath, and the water kept gently boiling during the extraction process, which should last for two hours. At the end of that time, the contents of the flask are cooled to about 15° and made up to the mark with cold distilled water. A total volume of 508 c.c. is taken, as it was found by experiment that the average volume occupied by 10 grm. of hops is

8 c.c. The liquid is then filtered through a dry filter paper into a dry beaker, and 50 c.c. of the clear filtrate are evaporated slowly in a small beaker, which is allowed to stand on the top of a boiling water bath. When the liquid has been concentrated to 15 c.c. it is cooled and 50 c.c. of a saturated aqueous solution of cinchonine sulphate are added. It is well to allow the precipitate to stand for an hour or two in a cool place, after which it should be filtered through a weighed porcelain Gooch crucible containing a bed of prepared asbestos. Inasmuch as the precipitate is to be washed ultimately with ½% solution of cinchonine sulphate (prepared by diluting one volume of the saturated solution with an equal volume of water), the Gooch crucible should be rinsed with the same solution before being weighed in order to correct for any small quantity of the salt which might adhere to it, and the empty crucible should be dried to a constant weight in a steam oven. The precipitated cinchonine tannate is then poured into the crucible and allowed to filter—at first without the aid of a pump. When about half the liquid has run through, suction should be applied, and the whole of the precipitate transferred to the crucible. When all the liquid has filtered through, the precipitate is washed several times, with a 0.5% solution of cinchonine sulphate, the same solution being, of course, used for washing out the beaker. As a point of detail, it may be mentioned that care should be taken not to allow the precipitate to dry on the sides of the beaker, as it then becomes very difficult to remove. When the washing is completed, the suction is continued until the cake of precipitate is moderately dry, as is shown by its tendency to separate into several portions. The crucible with its contents is then dried in a steam oven, and weighed at intervals until the weight is constant. The weight of precipitate so obtained multiplied by 0.6 represents the amount of tannin in 1 grm. of hops, from which the percentage of course is obtained. This process presents no working difficulty, and the actual filtration does not take more than one hour. The following table shows some of the results which have been obtained with Continental and English hops of various kinds.

TANNIN PERCENTAGE IN VARIOUS HOPS

Description of hop	Weight of cinchonine tannate. grm.	Moisture per cent.	Tannin percentage on (1) Sample	Tannin percentage on (2) Dry hop
Choice Bohemian, 1907	0.068 0.066	11.48	4.02	4.54
Choicest Hallertau, 1907	0.069	10.76	4.14	4.64
Sun-dried Spalt, 1906	0.066	11.28	3.96	4.46
Choice Hallertau, 1906	0.054 0 055	10.96	3.30	3.70
Choice Bavarian, 1907 (Mountain)	0 057	10.94	3.42	3.84
Choice Bavarian, 1907	0.058 0.055	10.00	3.42	3.80
Choice Hallertau, 1907	0.063	11.08	3.78	4.24
Alsace, 1907	0.048	10.14	2.88	3.20
East Kent Goldings, 1907	0.038 0.035	15.10	2.28	2.68
East Kent Goldings, 1907	0.037 0.038	13.26	2.28	2.62
Mid Kent, 1907	0.035 0.037	14.06	2.16	2.51
Kent Fuggles, 1906	0.039	11.82	2.34	2.65
Mid Kent, 1905	0.039 0.040	10.40	2.40	2.67
Choice Worcester, 1905	0.035 0.035	10.76	2.10	2.35
Mid Kent, 1905	0.033 0.034	10.04	2.04	2.26
East Kent, 1907	0.032 0.034	14.24	2.00	2.32

The second column in the above table shows the weights of cinchonine tannate, whilst in the third column are given the percentages of tannin in the samples and in the fourth the percentage of tannin expressed on the dry hops. The numbers in the last column have been included so as to render the results more directly comparable. The numbers which are bracketed in the second column are intended to show the degree of concordance which may be expected in duplicate estimations. On reference to the above numbers it will be seen that the percentages of tannin expressed on the dry hops range from 2.26 to 4.64 per cent., the higher numbers being in all cases given by Continental hops.

Chapman's method has been tested in the writer's laboratory by D. Hooper (Analyst, 1925, **50**, 162), who has found it to give very

good results with a great number of tannins. Hooper finds that catechin can be estimated by the method in the presence of gallotannin, as cinchonine only precipitates the gallotannin.

The following cutches were analysed by Hooper, and his results are given below:

(1) Catechu from Cawnpore, U.P., Re 1½ per lb.; (2) Catechu from Surat, Gujerat, Re 1¼ per lb.; (3) Catechu in squares, Re 1 per lb.; (4) Catechu from Shirval, local product, generally used for dyeing, Re 1 per lb.; (5) Catechu in small square crystals, Re 1 per lb.; (6) Catechu powder from Burma for dyeing, 8 as. per lb.; (7) Catechu from Burma, blocks for dyeing, 8 as. per lb.

	1 Per Cent.	2 Per Cent.	3 Per Cent.	4 Per Cent.	5 Per Cent.	6 Per Cent.	7 Per Cent.
Water	11.2	7.0	2.8	10.0	9.2	7.0	10.0
Catechin	43.4	10.6	1.0	13.8	24.4	12.4	15.0
Tannin by cinchonine	16.6	4.5	3.0	44.0	23.0	17.1	44.5
Sol. non-tannins	13.6	5.1	1.8	20.6	21.0	16.9	24.9
Organic insol	5.6	25.8	13.4	9.6	8.2	13.4	3.3
Ash	9.6	47.0	78.0	2.0	14.4	33.2	2.3
	100.0	100.0	100.0	100.0	100.0	100.0	100.0

The catechin was separated and determined by crystallisation from an aqueous solution of the alcoholic extract, the mother liquors being evaporated, and the catechin separated as long as any crystals formed. This method was used by Hooper in finding the percentage of catechin in a large number of samples of cutch examined in India (*Agricultural Ledger*, No. 3, 1906). The figures in the present instance were confirmed by evaporating and separating the crystals from the aqueous solution of the ethyl acetate extracts.

There appears to be no definite ratio between catechin and tannin in these commercial samples of cutch. In 1 and 2 the catechin is more than double the amount of tannin, and the proportion is reversed in 4 and 7; in No. 5 the catechin and tannin are nearly equally balanced. The results of the hide powder method are not given in the above table since they include catechin. It must be conceded that the analysis showing the catechin separated in a crystalline condition, and the tannin determined by cinchonine, shows the composition of cutch in a better way than the usual method of analysis.

F. Jean (*Bull. Soc. Chim.*, 1885, (ii), 44, 183) has described a process of estimating tannin, based on the volume of the infusion requisite to tender a solution of an iron salt opaque. The operation is conducted in a beaker 8.5 cm. in diam., placed in a good light on a black cloth, having on it a small circle of white paper about 5 cm. in diam. 5 c.c. of a solution of iron, containing 14 grm. of ferric chloride and 10 c.c. of hydrochloric acid per litre, are run into the beaker and 200 c.c. of water added. A 0.1% solution of tannin is then dropped in with constant stirring. The titration is finished when the disc of white paper is wholly invisible after the liquid has come to rest, which in the case of pure gallotannin occurs when 11.6 c.c. of the solution have been added. In comparing tanning materials with this it is simply necessary to take care that the infusions are approximately of the same richness in tannin, and this may be attained by extracting 1.5 grm. of European bark, 1.0 of African bark, 0.5 of quebracho, 0.5 of sumach, or 0.25 grm. of catechu, and diluting the liquid to 100 c.c. The estimation can be made very rapidly, and is said to be accurate to 0.5%. By subsequently repeating the experiment with a solution which has been treated with hide powder the error caused by gallic acid may be eliminated. This process might be useful in the dyer's laboratory for special work.

E. Durien proposed to estimate tannin by adding acetic acid and ferric chloride to the infusion, and then dropping in a standard solution of bleaching powder (5 grm. per litre) till the colour of the liquid changes suddenly to a rose-brown tint. Sugar was found not to affect the result, but gallic acid was not considered.

Musset (*Z. anal. Chem.*, 1884, **23**, 584) described a method of titrating tannin by oxidation with iodine. 100 c.c. of a 1% solution of bark are treated with 20 c.c. of N/10 solution of iodine (12.7 grm. per litre), the flask filled to the neck with warm air-free water, and carefully closed. After 12 hours, the free iodine is reduced by standard thiosulphate solution, which should be added somewhat in excess, and the liquid titrated back with N/10 iodine and starch. By operating in a similar manner on a solution which has been treated with hide powder, the error due to the presence of gallic acid and other "not tannin" matters is ascertained.

A. Moullade (*J. Pharm. Chim.*, 1890, **22**, 153) describes a method of estimating tannin by means of iodine in the presence of sodium hydrogen carbonate. Carbon disulphide is used as an indicator.

The iodine solution should contain 5.2 grm. of iodine and 7.6 grm. of potassium iodide per litre; the sodium bicarbonate solution is 1:10. To 10 c.c. of a tannin solution 30 c.c. of the bicarbonate solution are added, together with 2–3 c.c. of carbon disulphide. The iodide solution is introduced from a burette until a blue colour appears. Several titrations are necessary to ensure good results. In the presence of substances similar to tannin, two experiments are necessary, in one of which the tannin is precipitated by the gelatin; the difference between the 2 titrations corresponds to the tannin present.

According to Boudet (*J. Soc. Chem. Ind.*, 1902, **25**, 956) standard iodine solution is used in excess before and after detannising with hide-powder, the excess of iodide being estimated with thiosulphate.

Gardner and Hodgson suggest a modified iodine method (*Chem. Soc. Proc.*, 1908, **24**, 273), standard iodine solution being added in excess. Sodium hydroxide solution is added drop by drop until the colour disappears, and concentrated hydrochloric acid is then added to precipitate the unabsorbed iodine, which is estimated by thiosulphate solution. Gelatin is recommended to separate the tannin. (See also Proc. VII, Intl. Congress. Appl. Chem., 1909, Section I.)

The detection of gallic acid in the presence of tannins is said to be achieved by titrating with iodine in the presence of sulphuric acid as well as in its absence. Sulphuric acid is said by Grassler (*Collegium*, 1910, 406) to prevent the combination of iodine with gallic acid under these conditions.

Guenez (*Compt. rend.*, 1890, **110**, 532) gives the following volumetric method for the estimation of tannin. A standard solution is prepared containing 12 grm. of tartar emetic and 1 grm. of Poirier's green 4JE to 1 litre of water. The solution of tannin is run from a burette into 20 c.c. of the boiling coloured solution until it is completely decolorised. The standard solution may be standarised by a solution of pure oak-gall tannin of known strength. Gallic acid does not interfere with the process.

L. Roos (*J. Pharm. Chim.*, 1890, **22**, 59) gives a volumetric method adapted to the estimation of tannin in wines. A 10% solution of tartaric acid is used, made slightly alkaline with ammonia; neutral lead acetate is then added until the precipitate no longer dissolves, and then the solution is filtered. Tannin is said to be completely

precipitated by this solution, sodium sulphide being used as an indicator. About 25 c.c. of the wine are taken for analysis and made slightly alkaline with ammonia.

P. Wilhelm (*Rev. gen. des. mat. col.*, 1898, **11**, 307) described a method of estimating tannin by adding the tannin solution to a known volume of standardised methylene blue solution (containing a small quantity of ammonia to neutralise the mineral acid set free) until the action is complete. The methylene blue solution should contain 12.5 grm. dissolved in 1 litre of water, and the colouring matter should be free from zinc. The tannin solution is titrated into the methylene blue solution to which a little ammonia has been added. The end-point is ascertained by spotting from time to time on stout filter-paper. When all the blue has been precipitated, the back of the spotted filter-paper remains colourless. The process has, it is claimed, given results within 2%.

L. Vignon (*Compt. rend.*, 1898, **127**, 369) described a method for the estimation of tannin by the use of silk. He claimed that silk free from silk gum absorbs tannin readily and completely from solutions of tannin materials, but does not absorb gallic acid, dextrose etc. The tannin may be estimated either by the increase in weight of the silk, or by the difference in the proportion of solid matters in the solution before and after treatment with silk, or by titration with permanganate. The accuracy of this method, however, depends on the nature of the tannin material employed, as silk does not appear to absorb all tannin materials in the same proportion. It also absorbs gallic acid very readily under certain conditions.

Hinsdale (*Chem. News.*, 1890, **62**, 19) gave the following colorimetric method for estimating tannin in bark. The following solutions are prepared: Dissolve 0.04 grm. potassium ferricyanide in 500 c.c. water, and add to it 1.5 c.c. liquid ferric chloride; this is called the iron mixture. Dissolve 0.04 grm. "pure tannin" (gallotannin) which has been dried at 100°, in 500 c.c. of water; call this the tannin solution. 0.8 grm. of the bark is exhausted with boiling water and the extract made up to 500 c.c. with cold water. Place six 2-ounce beakers on a white surface, and in one of them place 5 drops of the bark infusion, and in the others put 4, 5, 6, 7, and 8 drops of the tannin solution. Add to each 5 c.c. of the iron mixture, and then make a further addition of 20 c.c. of water to each after about 1 minute, and

within three minutes observe the shades of colour. Then the number of drops of tannin solution used in the beaker which corresponds in shade of colour to the beaker containing the bark infusion indicates the percentage of tannin in the bark. The results are necessarily in terms of commercial gallotannin, and not in those of pure tannin or of the particular tannin in the material assayed. For substances containing over 10% of tannin, the infusion should be proportionately diluted.

Wislicenus (*Collegium*, 1904, 204) has suggested the substitution of a fibroid alumina in the place of the hide powder. It is said to give results which are of special value, although it is doubtful whether it will ever replace the latter in the official method, for the non-tannins are precipitated to a certain degree as well as the tannins, and possibly to a different degree from those attracted by hide powder. The alumina may be repeatedly used after ignition. It can be obtained in commerce.

Baum (*Collegium*, 1906, 373) detannises the solution in an aluminium vessel through which solution a slow voltage current flows, the aluminium tannate is formed and weighed directly.

The estimation of tannin by electrolytic methods has also been suggested by Metzges (*Collegium*, 1908, 259). 250 c.c. of the solution was submitted to a current of "longue" phase and 110 volts using aluminium anodes. After 30 minutes the tannins were entirely precipitated, and the non-tannins were estimated in 50 c.c. of the solution by evaporation.

Mitchell's colorimetric method (Vol. III p. 566) has been used for the quantitative determination of the degree of hydrolysis of gallotannin by tannase by Nicholson and Rhind (*Analyst*, 1924, **49**, 505), who have modified it as follows: The hydrolyses were carried out in a series of test tubes, into each of which were put 10 c.c. of a gallotannin solution, containing about 3 grm. per litre. To this was added 1 c.c. mycelium extract, prepared by shaking 1 grm. of mycelium powder with 50 c.c. of distilled water for 3 hours, allowing this to stand for 21 hours and filtering. A layer of benzene was poured on the surface to prevent growth of fungi. The test tubes were corked tightly and placed in an incubator at 25°, one being used for the gallic acid estimation every twenty-four hours. To precipitate the unchanged gallotannin 4.5 c.c. of a 1% solution of quinine hydrochloride were added, and 0.5 c.c. of a 16% solution of sodium

chloride, to coagulate the precipitate. One c.c. of this mixture was added to one of the Nessler tubes, and 1 c.c. of standard gallic acid and 1 c.c. of a 0.5% solution of sodium chloride to the other. To each tube 2 c.c. of Mitchell's ferrous tartrate reagent were added and the comparison of colour carried out as given by Mitchell. Nicholson and Rhind describe a special apparatus for the use of the Nessler tubes and reference should be made to the original. Reference should also be made to a paper by Glasstone (*Analyst* 1925, **50,** 49) on the influence of the hydrogen-ion concentration on Mitchell's reagent as applied to the pyrogallol and catechol tannins.

Other Quantitative Data on Tannin-Containing Materials

I. *Laufmann's Molybdenum Figures.*—Reference has already been made to this method (page 56). The following data are given by Laufmann for:

Not sulphited quebracho extract	28.7– 37.8
Slightly sulphited quebracho extract	36.9
Strongly sulphited quebracho extract	5.0– 21.3
Not sulphited mangrove extract	124.2–144.5
Sulphited mangrove extract	111.3
Mimosa extract	128.1
Chestnut-wood extract	195.3–202.3
Oak-wood extract	197 7–205 8
Myrobalans extract	108.0–122.2

II. *Laufmann's Acidity Figures.*—These are obtained as follows: 25 c.c. of the tannin solution are titrated with $\frac{1}{10}$N. KOH, phenolphthalein being used as indicator. A further 25 c.c. of the "detannised" tannin solution are titrated in the same way. The difference between the two titrations is calculated per grm. of tannin found by the official method. In this manner the following data, which are termed the "acidity values," were obtained (*Collegium*, 1920, 129):

Quebracho wood	92–114
Mangrove bark	39– 55
Malitto bark	76– 86
Mimosa bark	92
Oak bark	122–143
Chestnut wood	260–283
Oak wood	234
Myrobalans	257–261
Sumach	248–269
Valonia	218
Trillo	193–229
Gallotannin	222

III. *Stiasny's Solubility Values.*—This method has already been described (p. 76). The following data have been obtained by Stiasny:

	Alcohol-value	Ethyl acetate-value
Algarobilla	0–5	50–60
Divi-divi	0–10	30–50
Oak-wood	20–30	0–12
Oak bark	15–19	0–1
Gambier	5–10	50–65
Hemlock	8–10	16–20
Chestnut wood	10–20	0–16
Mangrove bark	0–5	0–5
Mimosa bark	0–5	30–40
Myrobalans	0–15	30–50
Quebracho wood	0–5	70–80
Sumach	5–20	40–60
Valonia	20–40	5–15

IV. *Bennett's Nitrogen Values* (*Collegium*, London 1916, 1).

	Nitrogen, %
Myrobalans	0.56
Valonia	0.29
Trillo	0.34
Mimosa bark	0.87
Sumach	0.87
Quebracho wood	0.17
Quebracho extract	1.9–2.6
Myrobalans extract	7.8–8.4
Chestnut wood extract	3.0
Hemlock extract	6.3
Cube gambier	10.8
Block gambier	16.4

V. *Kobert's Agglutination Values.* (*Ber. Deutsch. Pharm. Gesellsch*, 1914, **24**, 470; *Collegium* 1915, 321).

Pegu-cutch	1:1000
Oak bark	1:2948
Willow bark	1:3190
Algarobilla	1:3868
Maletto bark	1:5020
Pine bark	1:5555
Knoppern	1:7143
Galls	1:7174
Quebracho wood	1:7342
Myrobalans	1:11110
Valonia	1:14444
Mimosa bark	1:15151
Divi-divi	1:19802
Sumach	1:20833
Gallotannin	1:25000
Mangrove bark	1:30300

For further details as to the possible application of this method to the quantitative estimation of tannin reference should be made to the above-quoted papers by Kobert.

DETECTION AND ESTIMATION OF GLUCOSE IN TANNING MATERIALS

As the quantities found are often very small, it becomes desirable somewhat to vary the methods adopted in ordinary sugar estimation, so as to avoid the necessity of concentration and the use of inconveniently large quantities of materials. The subject has been investigated by Simand (*Zeitsch. angew. Chem.*, 1892, Heft. 22) and by v. Schroeder, Bartel and Schmitz-Dumont, (*Ding. polyt. J.*, 293, 1894, 229) and the methods given are selected from those they describe. They are modifications of that originally devised by Fehling.

Preparation of Fehling's Solution.—This is best prepared in two separate solutions which are only mixed at the time of using. It is suitable either for Fehling's original volumetric method, or for the gravimetric process of Allihn and its modification adopted by v. Schroeder.

(1) 34.639 grm. of the purest crystallised copper sulphate are dissolved in distilled water, 10 c.c. of normal sulphuric acid are added, and the whole made up to 500 c.c.

(2) 173 grm. of pure crystallised potassium sodium tartrate (Rochelle salt) and 125 grm. of potassium hydroxide "pure by alcohol" are dissolved in water and made up to 500 c.c.

Von Schroeder's Gravimetric Method.—This is a modification of Allihn's method specially adapted for the small quantities of sugars naturally found in tanning materials and leathers. As the result is to a marked extent dependent upon the concentration of solutions and the time of heating, the details given must be rigidly adhered to in order to get accurate results, and the special table of Koch and Ruhsam given by v. Schroeder must be employed.

Preparation and Decolorisation of Solutions.—It is necessary to remove all tannin and gallic acid as well as colouring matters. This is best accomplished with basic lead acetate. The lead solution is prepared by rubbing together 300 grm. of pure lead acetate, 100 grm. of pure, finely powdered litharge, and 50 c.c. of water, and digesting

the mass on the water-bath till it becomes white, water being added as required to replace evaporation. The mass is made up to 1 litre with distilled water, allowed to settle and filtered.

10 c.c. of the lead solution are titrated with a strong solution of sodium sulphate till no further precipitate is produced, and 100 times the quantity of sulphate solution so employed is made up to 1 litre. The titration need not be very accurate, but it is better to have the sulphate a little in excess than the reverse. Sodium sulphide may, if required, be used as an indicator by "spotting" it on filter-paper.

Estimation.—30 c.c. of each of the Fehling's solutions and 60 c.c of water are put into a beaker of 200 c.c. capacity and heated to boiling over a flame. The beaker is then placed in a boiling water-bath, 25 c.c. of the decolorised sugar solution are introduced and stirred and maintained at boiling temperature for exactly 30 minutes. If the sugar solution is very weak, 50 or 75 c.c. may be used, the water added to the Fehling's solution being correspondingly reduced so that the total volume remains 145 c.c.

After 30 minutes the liquid is allowed to settle and at once filtered through a weighed asbestos filter with the aid of the filter-pump. The filter consists of a piece of combustion tube, about 10 cm. long and 1–2 cm. interior diameter. The lower end is drawn out to about half of its original diameter, and a small funnel is fitted into the top through a perforated rubber cork. A small plug of glass wool is placed in the conical part to prevent fibres of asbestos being carried through, and about 3 cm. of the tube are filled with fibrous asbestos, of which the lower part is pretty closely packed, while the upper part is quite loose. Packed in this way, the filter is much less liable to choke, as a large part of the cuprous oxide is caught on the loosely packed layer. As some samples of the asbestos are somewhat soluble in the solutions employed, it is well to wash the asbestos before use with hot 20% solution of sodium hydroxide, and then with distilled water and to make a blank experiment with a filter to ascertain that its weight remains constant.

The precipitate is thoroughly washed with hot water, and then with alcohol, and finally with a little ether to quicken the drying, which may be accomplished in an air bath in less than 15 minutes. The tube is then gently ignited with a slight current of air through it to destroy possible traces of organic matter and the copper reduced in a stream of pure dry hydrogen, the tube being heated by a Bunsen

burner. The reduction takes place at a low temperature, and it is not necessary to bring the tube actually into contact with the flame, and the glass wool especially must not be ignited. When the precipitate has taken the colour of metallic copper it is allowed to cool in the stream of hydrogen. A little dry air is now sucked through to expel the hydrogen, and the tube is weighed; from the gain in weight the amount of glucose is found by means of the table on pp 194-196. The same filter may be repeatedly used if the copper be removed with a few drops of strong nitric acid, and the filter well washed with water, alcohol and ether, dried and re-weighed. Under this treatment a good filter should not lose any appreciable weight.

In place of using the asbestos tube and reducing the oxide to metallic Cu, small ordinary quantitative filter papers may be employed, preferably with a perforated platinum (or parchment-paper) cone in the funnel and with the aid of the vacuum-pump. As the Cu_2O is very finely divided, it is sometimes necessary to use a double filter. The precipitate should be first washed repeatedly by decantation to free it as far as possible from the alkaline solution, and afterwards on the filter till the latter no longer reacts with phenolphthalein. Paper absorbs and obstinately retains traces of copper, and Fehling's solution sometimes gives a slight precipitate when boiled alone, so that it is necessary to determine these errors by a blank experiment without sugar, and use the result as a correction. The paper is dried in a basin or crucible, slowly charred and heated for some time, first to a dull, and afterwards to a bright red heat, and the precipitate is weighed as CuO, which must be reduced to Cu for comparison with the table by multiplication by $\frac{63.6}{79.6}$ or practically by 0.8. If complete oxidation is difficult, the precipitate may be moistened with concentrated nitric acid and again ignited.

Hellon, Winter Blyth and others, redissolve the copper oxide in dilute nitric acid and deposit electrolytically on a platinum dish, a continuous current of ½ to 1 ampere current-density and 2.2 to 2.5 volts being used. The solution (100 c.c.) should not contain more than three per cent. of nitric acid, and the temperature should be 20 to 30° C. When all the copper has been deposited, which will require from 2 to 5 hours, and may be tested by the addition of ammonia to the solution, the dish is washed with distilled water

while the current is still passing, till all nitric acid is removed. The deposited copper is now washed further under the tap, rinsed with methylated alcohol and then with ether, dried in a water oven, cooled, and weighed, the basin being afterwards freed from copper with nitric acid. An ordinary electric supply may be used of continuous current, the resistance being regulated by lamps. The anode is a piece of platinum foil, and the basin, forming the cathode, is connected with the negative wire and is conveniently placed in a photographic tray to receive the washings.

The sugar naturally present in tanning materials is not merely important as influencing the calculation of weighting by glucose, but as furnishing the basis from which the natural acids of the liquors are derived by fermentation. The sugar is of more than one kind, dextrose, lævulose and less definitely known sorts being present in different materials, but probably the estimation of total sugars as glucose by Fehling's solution gives a pretty accurate idea of their total percentage. The result of a large number of estimations by Von Schroeder (*Gerberei-Chemie*, Berlin, 1898, 577–623), are given in the following table. These estimations were made by the gravimetric method after removal of the tannin by precipitation with lead and therefore only include the sugars existing as such ready formed in the infusion. In addition to these, it is known that many tannins, like the glucoside colouring matters, contain sugars or sugar-yielding bodies, and like these can be hydrolysed by digestion with dilute acids. Procter, however, has been unable to find in literature detailed observations either with regard to the quantities of these sugars, or the time, temperature and concentration of acid necessary for complete hydrolysis. If the heating with acid, such as is described for conversion of starch into glucose, were carried out before, instead of after, the detannisation with lead, any sugars liberated from the tannins would, of course, be estimated with those existing ready formed.

TABLE FOR THE ESTIMATION OF THE GLUCOSE IN TANNING MATERIALS FROM THE WEIGHT OF COPPER REDUCED, AFTER HEATING FEHLING'S SOLUTION WITH THE GLUCOSE SOLUTION FOR HALF AN HOUR

(R. Koch and R. Ruhsam)

Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.
1	0.4	46	19.3	91	42.3	136	65.1
2	0.8	47	19.7	92	42.8	137	65.6
3	1.2	48	20.2	93	43.3	138	66.1
4	1.6	49	20.7	94	43.9	139	66.6
5	2.0	50	21.3	95	44.4	140	67.1
6	2.5	51	21.8	96	44.9	141	67.6
7	2.9	52	22.3	97	45.4	142	68.1
8	3.3	53	22.8	98	45.9	143	68.6
9	3.7	54	23.3	99	46.4	144	69.1
10	4.1	55	23.9	100	46.9	145	69.6
11	4.5	56	24.4	101	47.5	146	70.1
12	4.9	57	24.9	102	48.0	147	70.6
13	5.3	58	25.4	103	48.5	148	71.1
14	5.7	59	25.9	104	49.0	149	71.5
15	6.1	60	26.4	105	49.5	150	72.0
16	6.5	61	26.9	106	50.0	151	72.5
17	7.0	62	27.4	107	50.5	152	73.0
18	7.4	63	28.0	108	51.0	153	73.5
19	7.8	64	28.5	109	51.6	154	74.0
20	8.2	65	29.0	110	52.1	155	74.5
21	8.6	66	29.5	111	52.6	156	75.0
22	9.0	67	30.0	112	53.1	157	75.5
23	9.4	68	30.5	113	53.6	158	76.0
24	9.9	69	31.0	114	54.1	159	76.5
25	10.3	70	31.6	115	54.6	160	77.0
26	10.7	71	32.1	116	55.1	161	77.5
27	11.1	72	32.6	117	55.7	162	78.0
28	11.6	73	33.1	118	56.2	163	78.5
29	12.0	74	33.6	119	56.7	164	79.0
30	12.4	75	34.1	120	57.2	165	79.5
31	12.9	76	34.6	121	57.7	166	80.0
32	13.3	77	35.1	122	58.2	167	80.5
33	13.7	78	35.7	123	58.7	168	81.0
34	14.1	79	36.2	124	59.2	169	81.4
35	14.6	80	36.7	125	59.7	170	81.9
36	15.0	81	37.2	126	60.2	171	82.4
37	15.4	82	37.7	127	60.7	172	82.9
38	15.9	83	38.2	128	61.2	173	83.4
39	16.3	84	38.7	129	61.7	174	83.9
40	16.7	85	39.2	130	62.2	175	84.4
41	17.2	86	39.8	131	62.6	176	84.9
42	17.6	87	40.3	132	63.1	177	85.4
43	18.0	88	40.8	133	63.6	178	85.9
44	18.4	89	41.3	134	64.1	179	86.4
45	18.9	90	41.8	135	64.6	180	86.9

TABLE FOR THE ESTIMATION OF THE GLUCOSE IN TANNING MATERIALS FROM THE WEIGHT OF COPPER REDUCED, AFTER HEATING FEHLING'S SOLUTION WITH THE GLUCOSE SOLUTION FOR HALF AN HOUR.—(*Continued*)

(R. Koch and R. Ruhsam)

Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.
181	87.4	226	110.2	271	133.7	316	157.6
182	87.9	227	110.7	272	134.2	317	158.1
183	88.4	228	111.2	273	134.7	318	158.7
184	88.9	229	111.8	274	135.3	319	159.2
185	89.4	230	112.3	275	135.8	320	159.8
186	89.9	231	112.8	276	136.3	321	160.3
187	90.4	232	113.3	277	136.8	322	160.9
188	90.9	233	113.8	278	137.4	323	161.4
189	91.3	234	114.4	279	137.9	324	162.0
190	91.8	235	114.9	280	138.4	325	162.5
191	92.3	236	115.4	281	139.0	326	163.0
192	92.8	237	115.9	282	139.5	327	163.6
193	93.3	238	116.4	283	140.0	328	164.1
194	93.8	239	117.0	284	140.5	329	164.7
195	94.3	240	117.5	285	141.1	330	165.2
196	94.8	241	118.0	286	141.6	331	165.8
197	95.3	242	118.5	287	142.1	332	166.3
198	95.8	243	119.0	288	142.6	333	166.9
199	96.3	244	119.5	289	143.2	334	167.4
200	96.8	245	120.1	290	143.7	335	167.9
201	97.3	246	120.6	291	144.2	336	168.4
202	97.8	247	121.1	292	144.7	337	169.0
203	98.3	248	121.6	293	145.3	338	169.5
204	98.8	249	122.1	294	145.8	339	170.1
205	99.3	250	122.7	295	146.3	340	170.6
206	99.8	251	123.2	296	146.9	341	171.2
207	100.3	252	123.7	297	147.4	342	171.7
208	100.8	253	124.2	298	147.9	343	172.2
209	101.4	254	124.8	299	148.4	344	172.8
210	101.9	255	125.3	300	149.0	345	173.3
211	102.4	256	125.8	301	149.5	346	173.9
212	102.9	257	126.3	302	150.1	347	174.5
213	103.5	258	126.9	303	150.6	348	175.0
214	104.0	259	127.5	304	151.1	349	175.6
215	104.5	260	128.0	305	151.7	350	176.2
216	105.0	261	128.5	306	152.2	351	176.8
217	105.5	262	129.0	307	152.8	352	177.3
218	106.0	263	129.5	308	153.3	353	177.9
219	106.6	264	130.1	309	153.9	354	178.5
220	107.1	265	130.6	310	154.4	355	179.1
221	107.6	266	131.1	311	155.0	356	179.6
222	108.1	267	131.6	312	155.5	357	180.2
223	108.7	268	132.2	313	156.0	358	180.8
224	109.2	269	132.7	314	156.5	359	181.4
225	109.7	270	133.2	315	157.1	360	181.9

TABLE FOR THE ESTIMATION OF THE GLUCOSE IN TANNING MATERIALS FROM THE WEIGHT OF COPPER REDUCED, AFTER HEATING FEHLING'S SOLUTION WITH THE GLUCOSE SOLUTION FOR HALF AN HOUR.—(*Continued*)

(R. Koch and R. Ruhsam)

Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.	Cu, mg.	Glucose, mg.
361	182.5	390	199.2	419	216.1	448	234.5
362	183.1	391	199.8	420	216.7	449	235.2
363	183.7	392	200.3	421	217.3	450	235.9
364	184.2	393	200.9	422	217.9	451	236.6
365	184.8	394	201.5	423	218.4	452	237.2
366	185.4	395	202.1	424	219.0	453	237.9
367	186.0	396	202.7	425	219.6	454	238.6
368	186.5	397	203.3	426	220.2	455	239.3
369	187.1	398	203.8	427	220.8	456	239.9
370	187.1	399	204.4	428	221.4	457	240.6
371	188.3	400	205.0	429	221.9	458	241.3
372	188.8	401	205.6	430	222.5	459	242.0
373	189.4	402	206.2	431	223.1	460	242.6
374	190.0	403	206.8	432	223.7	461	243.3
375	190 6	404	207.3	433	224.4	462	244.0
376	191.1	405	207.9	434	225.1	463	244.7
377	191.7	406	208.5	435	225.8	464	245.3
378	192.3	407	209.1	436	226.4	465	246.0
379	192.8	408	209.7	437	227.1	466	246.7
380	193 4	409	210.3	438	227.8	467	247.4
381	194.0	410	210.8	439	228.5	468	248.0
382	194.6	411	211.4	440	229.1	469	248.7
383	195.2	412	212.0	441	229.8	470	249.4
384	195.7	413	212.6	442	230.5	471	250.1
385	196.3	414	213 2	443	231.2	472	250.8
386	196.9	415	213.8	444	231.8	473	251.4
387	197.5	416	214.4	445	232.5	474	252.1
388	198.0	417	214.9	446	233.2	475	252.8
389	198.6	418	215.5	447	233.9	476	253.5

Von Schroeder made a number of estimations of sugars in extracts, and in many cases the results show a higher percentage than is found in the original material. It is not certain that such a result indicates adulteration with glucose, which is rarely practised.

SUGARS NATURALLY CONTAINED IN TANNING MATERIALS
(Von Schroeder)

	Tanning matter	Sugars % on Material	Sugars % on Tanning matter
Oak bark (average 118 samples)	10.5	2.7	25.2
Oak bark inner flesh, tree 150 years	13.8	1.3	9.5
Oak bark outer crust, tree 150 years old	7.6	0.7	9.2
Oak bark, young tree bark (Tharand)	13.0	6.6	50.8
Oak wood, trees over 100 years old (Mitrowitz)	7.7	0.4	5.8
Oak wood, *Q. sessiliflora*. Young oak, 19 years; wood only	2.2	1.2	53.7
Evergreen oak. *Q. ilex*, bark	17.7	3.6	20.3
Garouille. Root-bark *Q. coccifera* average	25 4	1.0	4.0
Pine bark, *Abies excelsa*, Lam., average	11.6	3.5	33.5
Willow barks (Russian), average	13.4	4.5	33.6
Mimosa barks (Australian wattles), average	28.4	0.9	3.2
Aleppo pine ("Scorza rossa"), outer bark	20.6	2.0	9 9
Hemlock pine, *Abies canadensis*, old bark	12.3	7.1	5.8
Divi-divi pods, *Cæsalpinia coriaria*	40.7	8.4	20.5
Algarobilla pods, *C. brevifolia*, average	42.9	8.2	19.1
Myrobalans fruit of *Terminalia chebula*, average	30.8	5.4	17.4
Valonia sugar very variable, average	28 3	2.7	9.5
Sumach (Sicilian)	27.8	4.6	16.6
Canaigre, root of Rumex hymenosepalus	30.1	4.3	14.3
Chestnut wood, without bark	8 3	0.3	2.9
Quebracho wood, Loxopterygium Lorenzi	24 4	0.25	1.0
Cube gambier, *Nauclea gambir*	47.2	1 9	3.9
Cutch, *Acacia catechu*, wood extract	39.9	0.5	1.3

Estimation of Colour in Extracts.—It is noticed in practice that a brightly coloured extract produces a light coloured bath and a good tannage. The colour can be tested by the optical or empirical method. In the first case, Lovibond's tintometer can be used, and a permanent record of the shade can be kept (See Vol. VI). This method is satisfactory for the testing of extracts during manufacture, but is not so valuable when the resulting shade obtained on leather is the consideration. The second method of testing has been improved by Eitner (*Gerber* 1910, **36,** 321), an "animalised fabric" being substituted for a piece of pelt which under old conditions was tanned under known conditions.

The animalised cotton is prepared in the following manner: A cotton material felted on one side, and 1 m.m. thick and 11 cm.

broad, is washed in boiling water, pressed and put on reels. This is then run through a 0.25% solution of formaldehyde and then through a 6% solution of gelatin, and subsequently dried in a protected position.

The extract to be tested is made up to 6° Bé., and 5 grm. of the animalised fabric are placed in it for a few minutes, and then churned for 12 hours. After being washed in water for 10 minutes and being squeezed, it is dried very slowly at a temperature not exceeding 30°. This process is said to give uniform results and to give a good indication of the value of the extract, so far as colour is concerned. It is possible that raw or gum silk would give similar results when substituted for the animalised fabric. The writer has used it for this purpose when the colour of the extract is to be tested for dyeing purposes.

Procter (*J. Soc. Chem. Ind.*, 1910, **29**, 663) suggests a variation in the method of colour measurement, and discusses the Schmidt and Haensch and the Laurient and Dubosc types of tintometer.

In testing the colour of extracts by the Lovibond method it seems that the temperature of solution has a great influence on the result obtained. Lamb (*Collegium*, 1910, 29) suggests that this error should be reduced by always dissolving the extract at a temperature of 60°.

English chemists have recently adopted a colour measurement in addition to that of the ordinary tintometer figures. The standard colour represents a proportion of red to yellow (with a necessary small correction of blue). The standard strength is the quantity of extract (or tannin) per 1000 parts of solution necessary to give the standard colour in a cell of 1 cm. thickness.

Examination of Tan-liquors.—Besides estimating the tannin and oxidisable substances in tan-liquors, it is desirable to obtain further information as to the proportion and nature of the free acids present. The acid is usually acetic acid, though butyric, lactic, and other acids produced by fermentation are frequently present. By titrating the liquor with lime water, (Methyl-orange as an indicator) the proportion of strong acids capable of producing "plumping," or swelling of the leather, will be roughly ascertained. Sulphuric acid is sometimes added for this purpose.

Hoppenstedt (*J. Amer. Leather Chem. Soc.*, 1906, **1**, 192) estimates free acid in tan-liquors by precipitating the tannin with quinine.

200 c.c. of diluted liquor taken, mixed thoroughly with 20 c.c. of a solution of 15 grm. of pure quinine in 110 c.c. of 95% alcohol, and filtered, and 100 c.c. titrated with N/10 sodium hydroxide, phenolphthalein being used as indicator, as the soluble salts formed by quinine and free acids react acid to this indicator. Results are calculated as free acetic acid.

This matter has been studied in detail by Bennett and Wilkinson (*Collegium*, 1907, **289**, 441), and the conclusion come to is that no process at present known will give absolute results. The Procter lime-water method seems to give results which are useful in practice. 10 c.c. of the filtered solution to be tested are titrated with a saturated solution of lime water until a permanent turbidity is obtained, due to the formation of an insoluble calcium tannate when the free acid is neutralised. This is the only method which does not involve the removal of the tannin from the solution. In case the special tannin present does not give an insoluble calcium salt it is better to add a little pure tannin to the solution. The lime water is standardised against N/10 hydrochloric acid solution, but Procter expresses the results in terms of acetic acid. The presence of boric acid and gallic acid interferes with the value of the results obtained, so that in practice the only use that the process can claim is in estimating the acids present which will give soluble calcium salts in terms of CaO. This is of value in practice, for the part played by the acids present is to form soluble salts with the alkali of the limed hide.

The quinine method of Hoppenstedt is not favourably reported on for this purpose by these investigators. The gelatin method of Koch, in which the tannin is removed by means of salted gelatin is of little value, as such coagula carry down other acids with avidity.

Bennett and Walker suggest a method in which lead oxide (3 grm.) is digested with 100 c.c. of the acid-tan liquor. All the tannins and the gallic acid and similar substances are precipitated, as well as sulphuric, boric, oxalic or carbonic acids present, so that only such acids as acetic, formic, and lactic acids are left in the solution; after filtering, 20 c.c. are titrated with N/10 potassium ferrocyanide in the presence of an excess of acetic acid, uranium acetate being used as an outside indicator which gives a brown coloration. Another 20 c.c. are taken, and an amount of N/10 sulphuric acid, equal to that required in the above titration added. A quantity of sodium

sulphate is then added, and the mixture warmed. The organic acids are thus liberated, and are estimated by N/10 potassium hydroxide, phenolphthalein being used as an indicator.

Grasser (*Collegium*, 1910 406) suggests an apparatus for estimating the acid present in tannin liquors. The carbon dioxide present is first removed by passing a current of air (free from that gas) through the liquor, absorbing in potassium hydroxide and weighing. Acetic and other volatile acids are then boiled and titrated with N/10 alkali. The residual acids (lactic acid, etc.) of a non-volatile nature are titrated with baryta and phenolphthalein after detannisation with gelatin solution (Koch's method). It must be remembered, however, that the gelatin coagulum will carry down with it free acid.

Procter and Seymour-Jones (*Collegium*, 1910, 299) have thrown doubt upon all the present methods of estimation, and they fall back on the direct titration of the liquor by N/10 sodium hydroxide. They rightly point out that detannisation by gelatin or hide powder removes other acids as well. The indicator used is fluorescein. This indicator fluoresces in alkaline solution, and this property is used to indicate the end-point. The indicator only comes into play at a hydrion concentration of 10^{-6}, and consequently only the acids which actually plump the skins are estimated.

Gum arabic has been suggested as a precipitant for both gallic acid and tannin from tannin liquors when the acidity is to be estimated, but the process has not been accepted as a better one than the gelatin separation.

F. Andreasch (*Der Gerber*, **23**, 111), in a study of the fermentation phenomena in tan liquors, showed that the acidity of the liquor is due to the following causes:

1. Putrefactive bacteria from the hides, bates, etc., accommodate themselves to the acid reaction of tan liquors; they dissolve certain nitrogenous constituents of the hide, and thereby furnish the chief nutriment for the more specific acid-producing bacteria. In liquors which are in use, the production of acid is proportional to the hide substance present, provided sufficient quantity of carbohydrates are present.

2. Acetic acid, which in fresh tan liquors is the chief acid, is always formed by two separate processes: (1) the production of alcohol by yeasts from the sugars of the non-tannins; and (2) the fermenta-

tion of the alcohol by acetic bacteria. In tan liquors it is never formed directly from carbohydrates.

3. Lactic acid is produced by several species of bacteria both from the sugars and other carbohydrates of tan liquors, and from the sugars alone by a yeast. A good supply of nitrogenous nutriment is necessary for its production, the greater part of which is furnished by the hides.

4. Butyric acid occurs only in satisfactory tan liquors.

Kohnstein and Simand (*Dingl. polyt. J.*, 1885, **38**, 256) estimated the *volatile organic acids* (acetic, butyric, etc.), as follows: 100 c.c. of the liquor are distilled to 30 c.c., the residue made up with water to the original bulk and again distilled, and the process repeated till the total distillate measures 300 c.c., when it is titrated with standard alkali hydroxide and phenolphthalein, and the acidity expressed in terms of acetic acid. By adding sulphuric acid and water to the contents of the retort, again distilling, and titrating the distillate, the combined acetic acid may be estimated.

Another portion of the liquid (80 or 100 c.c.) is shaken with 3 to 4 grm. of freshly ignited magnesia, quite free from carbonates and calcium. The mixture is left for some hours, with frequent agitation, when the brown or dirty green colour will have disappeared, and the filtered liquid will be nearly colourless, neutral, and free from tannin. The magnesia in solution is estimated in an aliquot part of the filtered solution, and will be equivalent to the total free acids of the liquor, exclusive of the tannic acid, which is completely precipitated together with the colouring matter. Another portion of the filtrate is evaporated to dryness, and the residue *gently* ignited. The ash is moistened with carbonic acid water and dried. It is then boiled with distilled water, and the solution filtered. The magnesia remaining insoluble corresponds with that which existed in the solution as magnesium salts of *organic acids*, and may be estimated gravimetrically as pyrophosphate, or dissolved in standard acid and titrated with alkali, with methyl orange or litmus as indicator. By dividing the percentage of acetic acid previously found by 3, and subtracting this figure from the percentage of MgO, the weight of the latter corresponding to the *non-volatile organic acids* of the liquor will be found; and 4.5 times this amount will be their equivalent of lactic acid. The magnesia contained in the aqueous solution of the ash is equivalent to the free *sulphuric acid* originally present.

The liquors of a set of seven handlers, in a Continental upper-leather tannery in which larch-bark was used, showed, by the above process in grm. per 100 c.c.: Total acids reckoned as acetic, from 0.20 to 0.68; volatile acids, 0.05 to 0.46; and fixed organic acids reckoned as lactic acid, 0.05 to 0.59.

The hide powder process for the gravimetric estimation of tannin is not applicable to the testing of acid liquors, because the hide absorbs a certain proportion of the acid, which is estimated as tannin.

J. Passler removed the greater portion of the volatile acid by repeated evaporations; the hide powder process can then usually be applied to the testing of tan-liquors containing acetic and lactic, and similar acids, without serious error; the results are sufficiently accurate for due control of the tanning process, and are said to be more accurate than those obtained by the Löwenthal or Murkatz processes. Such evaporation must clearly be conducted *in vacuo.*

Bacterial action produces considerable changes in the composition of tannin liquors, and a list of the chief of these will be found in an article by Wood (*J. Soc. Chem. Ind.*, 1910, **29**, 671).

Detection of Adulteration in Sumach and Extracts.—Book-binding leathers tanned with sumach (*Rhus coriaria*) are less likely to be affected by light, gas fumes, or to decay or "rot," so that adulteration is here particularly detrimental. This satisfactory condition is probably due to the absence of catechol tannins in the pure article. Many species of the *Rhus* family are used to adulterate sumach, but *Pistacia lentiscus* is chiefly used. Procter's bromine water test or a microscopical examination is the best way of detecting these leaves and twigs. Nierenstein and Webster (*Collegium*, 1907, 244) suggest the use of the diazobenzene chloride test. 5 grm. of the sumach are heated for about 6 hours and filtered. 10 c.c. of the extract are placed in narrow beakers and 10 c.c. of a 2% solution of diazobenzene chloride added, and the mixture allowed to stand 12 hours with as little exposure to air as possible. The precipitate is filtered and washed with dilute hydrochloric acid, then with distilled water, and the nitrogen estimated by Kjeldahl's method.

Sumach is also adulterated with leaves of *Tamarix africana.* In order to detect these adulterations the use of the following property of a pure sumach decoction has been suggested: If lead acetate in potassium hydroxide is added to a decoction of sumach and the

mixture concentrated, a brownish-red liquor is obtained, which assumes a claret colour when diluted with water. The intensity of this colour will depend on the amount of sumach present, and since the decoctions of the leaves used for adulteration do not give this colour, the reaction may be employed not only for their detection, but also for their quantitative estimation by colorimetry (Spica, *Gazetta*, 1897, **27**, 349).

For this purpose 5 grm. of the sample are boiled for half an hour in 500 c.c. of water. After cooling, the liquid is made up to its original volume and filtered. 25 c.c. of the filtrate are run into a flask, together with 5 c.c. basic lead acetate (sp. gr. 1.184, containing 250 grm. basic lead acetate per 1000 c.c.), and 15 c.c. potassium hydroxide solution (sp. gr. 1.155). The mixture is shaken and then boiled until the volume has decreased to 15 c.c. In the case of pure sumach, the concentrated reddish-brown liquid is almost perfectly clear. The presence of an insoluble precipitate is sufficient to indicate the probability of adulteration. To obtain the amount of adulteration the liquid is diluted to 250 c.c., filtered and examined colorimetrically. The intensity of colour of pure sumach is equal to that of a solution of 0.15 grm. of safranin in 1000 c.c. water, which may be taken as a standard in case a pure sumach sample is unavailable. Any suitable colorimeter may be employed for the estimation.

Spica also furnishes the following method of estimating the presence of *Pistacia lentiscus* in sumach: 0.5 grm. of the sample is boiled in a test-tube with 5 c.c. of an 18% solution of potassium hydroxide. Pure sumach gives a brownish-yellow colour becoming paler when diluted with water. If *lentiscus* is present the solution assumes a brown colour with a violet tint and on dilution this changes to a chestnut brown.

As the ash of *Tamarix africana* contains sulphates, their presence may be detected in the following manner: 1 grm. of the sample is boiled for half an hour with 100 c.c. of water; the filtered liquid is acidified with nitric acid, and barium chloride is added. It is said that if Tamarix is present the solution will become turbid.

F. Andreasch (*Gerber*, 1898, **24**, 573) gave the following method for the analysis of sumach containing adulterants from 8 to 60%: About 20 grms. of material are treated with a litre of water at 60° and filtered. The addition of several drops of formaldehyde (40% solution) gives a light yellow flocculent precipitate if *Pistacia* is present;

care must be taken to have the solution neutral. Arsenious acid solution, when warmed with a solution of *Pistacia*, gives a white precipitate; mercurous nitrate gives a yellow precipitate, which gradually turns green. A pure sumach should give no precipitate with formaldehyde. Sulphurous acid and potassium cyanide give no indication with pure sumach; if *Tamarix* is present, however, sulphurous acid produces a white precipitate or cloudiness; and potassium cyanide gives a dirty yellow precipitate. Sicilian sumach should never have less than about 22% of tannin, and not more than 18% of non-tannins. As the tannin in *Pistacia* and *Tamarix* is said to range from 8 to 17% and the non-tannins from 20 to 26%, a sample of commercial sumach should not contain less than 20% of tannin and more than 20% of non-tannins.

Myrobalans are used in blending extracts, although not so cheap a source as mangrove. Their presence can be detected by the Stiasny test (see page 76).

Dietrich (*Ber. Pharm. Ges.*, 1897, **7**, 153) states that an alcoholic solution of gambier (*Nauclea uncaria, Gambier*), when rendered strongly alkaline with sodium hydroxide imparts a strong green fluorescence to petroleum spirit when shaken with it.

The bark of chestnut-oak (*Q. prisnus*) exhibits a strongly blue fluorescence in alkaline solution. This is best seen after precipitating the tannin present with ammoniacal zinc solution.

The adulteration of tannin extract is, according to Eitner (*Gerber*, 1907, **61**, 700), chiefly confined to quebracho and mimosa. Chestnut-oak and hemlock are not often adulterated. Mangrove is used to adulterate quebracho. The former also is sometimes adulterated with grape-sugar. Mangrove is an inferior tannin material and may give trouble in the yard. Divi-divi and valonia extracts have been found to be adulterated.

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SECTION ON WRITING, STAMPING, TYPING AND MARKING INKS

BY C. AINSWORTH MITCHELL

INKS

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Modern writing inks are either solutions of pigments, or liquids containing a finely divided pigment in suspension.

The earliest writing inks were composed of lampblack or other form of carbon in admixture with a glue or gum, and were prepared for use by rubbing them on a palette with water. This type of ink is still used in the East, and is imported under the name of Chinese or Indian ink. Liquid carbon inks, in which the lampblack is in suspension, are still commercial products in Egypt and India (see p. 221).

The transition from carbon inks to iron-gall inks in Europe took place gradually from the tenth to the twelfth century. The early tannin inks were made by mixing a decoction of galls with copperas and gum and leaving the liquid exposed to the air until it yielded a mixture of colloidal and insoluble iron tannates which remained more or less in suspension in the liquid. This type of ink is now only exceptionally to be met with, for it has been replaced by the modern unoxidised iron-gall inks, which should remain as solutions of iron tannates until applied to the paper, when a gradual darkening will take place, with the formation first of colloidal and then of insoluble tannates, both on the surface and within the fibres of the paper.

As such ink by itself is too pale for use, a soluble pigment, such as indigo or an aniline dye, is added, to give the necessary colour pending the formation of the true pigment of the ink—the iron tannates. Originally madder was used for this purpose, whence the name "alizarine inks" which still survives, although this dye is no longer used for the purpose. Many of the so-called "blue-black" inks

contain a mixture of indigo and an aniline dye, which is frequently Soluble Blue.

In order to have a stable ink it is necessary to add the correct proportions of tannin substance and iron salt. Ferrous salts first yield a colourless solution with tannin, and this gradually changes to violet and then to bluish-black under the influence of oxidation, whilst ferric salts yield a blue-black compound immediately.

The materials used as sources of tannin in the manufacture of writing inks include Aleppo and Chinese galls, valonia, myrobalans (usually roasted), and (more rarely) sumach and oak bark. Commercial tannic acid (gallotannin) is also used, generally in admixture with gall extract, and gallic acid is a chief constituent of "acid-free" inks.

The value of these substances to the ink manufacturer obviously depends upon their tinctogenic power with ferrous sulphate, and this is best determined in terms of gallic acid by Mitchell's colorimetric method (*Analyst*, 1923, **48,** 2; see under **Gallic Acid,** Vol. III, p. 566).

Iron Salts.—The most important iron salt in the ink industry is ferrous sulphate or copperas ($FeSO_4.7H_2O$), which has long been used for this purpose. Theoretically it contains 20.08% of iron, but commercial samples may show variations ranging from about 18 to 25%, according to the method of preparation and the way in which they have been kept. On exposure to the air it becomes yellowish-brown owing to the formation of basic ferric sulphate, and in this condition is termed *rusty*, and is valued more highly by ink makers. As a rule it will be found that the price asked for the commercial salt is proportional to the percentage of iron present.

Commercial copperas is nearly always slightly acid (0.11N to 0.37N c.c. per 1 grm.). The acidity may be estimated by titrating a solution of the salt without an indicator, the end-point being shown by the production of ferrous hydroxide. Ferric chloride (crystalline and anhydrous) is sometimes used as the source of the iron in writing ink, and a ferric sulphate chloride, $FeSO_4\ Cl_2.6H_2O$, has also been recommended (*Chem. Zeit.*, 1921, **45,** 430).

Acid in Writing Inks.—Ordinary inks containing soluble and colloidal iron tannates rapidly change on exposure to the air, forming crusts and deposits of insoluble tannates. To render the ink stable in the bottle it is therefore necessary to add a sufficient proportion of a strong acid, but an excess is objectionable, since it retards the

darkening of the ink on the paper, and causes rapid corrosion of the pen nib. The methods of estimating the acidity of ink are described on p. 212. The acids generally used are hydrochloric, sulphuric and oxalic acids, in a proportion of about 0.1%.

Iron gallate solutions are much more stable than iron tannate solutions, and the addition of a strong acid to gallic acid ink is therefore not required.

Copying Inks.—Ordinary iron-gall inks will yield a poor copy shortly after writing, but not after the soluble tannate has changed into the insoluble forms. Commercial copying inks are made by using more concentrated solutions of the usual ingredients, and adding a substance to retard the drying process, *e. g.*, sugar, glycerin or calcium chloride. Roasted myrobalans are used as a source of tannin for copying inks.

Logwood Inks.—Hæmatoxylin, the colouring matter of logwood, combines with salts of iron to form an ink that is as permanent as iron-gall ink. It also combines with the salts of chromic acid to form deep black inks. The colour is modified by the addition of oxalic acid or alum. The objection to chrome-logwood inks is that they tend to gelatinise in the bottle. Logwood is sometimes added to ordinary iron-gall inks.

Vanadium Inks.—Ammonium vanadate forms a black ink with gallic acid or gallotannin, but the writing gradually turns brown on exposure.

Aniline Black Inks.—Various makes of nigrosine are used in solution as black writing inks, or as fountain pen inks. They lack the permanence of good iron-gall inks, and can usually be washed from the surface of the paper by means of a sponge.

Indulin ink (introduced in 1867) is a blue-black ink consisting of a solution or a mixture of aniline dyes.

Coloured Writing Inks.—The older coloured writing inks were composed of solutions of natural colouring matters or mineral pigments in solution or suspension. These have now, to a large extent, been replaced by aniline dyes.

The older formulæ included the following substances:

Red inks: Cochineal and ammonia; Brazil wood with acetic acid and alum.

Green Inks: Verdigris with cream of tartar; reduced potassium chromate.

Blue Inks: Prussian blue ground up with oxalic acid; indigo.

Violet Ink: Indigo and cochineal.

Purple Ink: Logwood extract with copper acetate and alum.

Aniline Dyes Used for Ink.—The following dyes are used in dilute solution as writing inks.

Red: Eosine, phloxine; ponceau scarlet; cotton scarlet.

Green: Neptune green S.G.; light green S.F.(yellowish); light green S.F. (bluish); diamond green.

Blue: Indigo carmine; soluble blue T.

Violet: Methyl violet; acid violet 4 B.L.

Yellow: Fast yellow; tartrazine. (See also Vol. VI, Artificial Dyes.)

Inks for Secret Writing.—In peace time these inks are mainly used as chemical playthings. They are preparations which remain invisible until heated or treated with a chemical substance which converts them into coloured compounds. For example, a dilute solution of potassium ferrocyanide is practically invisible on paper until treated with an iron salt. Similarly, a solution of lead acetate gives writing that may be developed with hydrogen sulphide; gallic acid with an iron salt; and so on.

A vanishing ink is composed of a decoction of starch tinted with iodine. The writing may be restored by exposing it to iodine vapour.

EXAMINATION OF WRITING INKS

It has been shown by Schluttig (*Lunge's Techn. Analysis*, Eng. Ed. 1914, p. 519) that useful information may sometimes be obtained by studying the behaviour of an ink when diluted with an equal volume of water and applied to filter paper.

Thus the zones formed as a drop spreads frequently vary with different kinds of ink. Chrome logwood inks yield a circular stain with a pale grey outer zone, which does not give the reaction for iron (blue on treatment with potassium bisulphate and ferrocyanide). Inks from galls and divi-divi give distinctive lines in the margin, whilst inks from sumach and myrobalans give an outer zone with a dark grey inner margin.

For the detection of *organic colouring matters*, a portion of the ink should be strongly acidified with hydrochloric acid. A blue colour, unaffected by the acid, but destroyed on adding bromine water or bleaching powder, shows the presence of indigo. If Prussian blue

is present, the ink will probably turn brown on addition of sodium hydroxide, and the filtered liquid will give a deep blue precipitate with ferric chloride, after being acidified with hydrochloric acid. A black colour, not destroyed by acids or alkalies, nor bleached by chlorine or bromine, is pretty certain to be due to finely-divided carbon. An ink prepared with ammonium vanadate and galls is turned blue by acids, but is unaffected by alkalies. Its colour is altered but not bleached by chlorine. Aniline black is not affected by alkalies, but is turned dark green by acids; bleaching powder renders it garnet-red. Logwood inks are turned red or yellow by hydrochloric acid, whilst those containing galls only are almost wholly decolorised by the same reagent.

Titration of an ink with a saturated solution of bromine water will often distinguish between different makes of blue-black ink; some turn grey and then greyish-black, whilst others change to violet and then brown, or violet and then green.

Composition.—Commercial iron-gall inks show considerable variations in the proportions of tannin material and iron salt, although the preparations of the same manufacturers keep fairly constant under normal conditions. Analyses of the principal kinds of ink on the English market will be found in the writer's book (*Inks and Their Manufacture*, p. 157). In these analyses the amounts of total solids range from 1.89 to 7.94%, the total mineral matter from 0.42 to 2.52%, and the iron in the iron-gall inks from 0.18 to 1.09%. Only one of the inks in the list is a chrome ink. It contains 1.22% of total solids and 0.26% of mineral matter.

Munson (*J. Amer. Chem. Soc.*, 1906, **28**, 28) examined 30 samples representative of the products of most of the important U. S. manufacturers. Only 3 of these were chrome logwood inks. In the iron-gall inks the total solids ranged from 1.26 to 16.87%, the mineral matter from 0.17 to 3.01%, and the iron from 0.05 to 1.80%.

The chrome-logwood inks contained 3.0 to 11.2% of total solids, 0.85 to 3.91% of mineral matter, and 0.06 to 0.74% of chromium (as $K_2Cr_2O_7$). A table is given showing the behaviour of these inks on paper when treated with water and with alcohol and on exposure to sunlight. They proved much less stable than most of the iron-gall inks.

Estimation of Iron.—The proportion of iron in writing ink is of great importance, since if too little is present, the ink will be deficient

in pigment and so will lack permanence. In the specifications of the British Stationery Office inks intended for record purposes must contain not less than 0.5% of iron, and fountain pen inks not less than 0.2%.

The Prussian regulations require documentary ink to contain not less than 0.4% or more than 0.6% of iron, and ordinary writing inks to contain not less than 0.26% or more than 0.4%.

The State of Massachusetts and the United States Government, have adopted the Prussian Standard and require Standard inks to contain 0.6% of iron.

The iron may be estimated either gravimetrically or volumetrically. In the former method the ash is dissolved in dilute hydrochloric acid, ammonia and hydrogen peroxide added, and the precipitated ferric hydroxide, filtered off, ignited and weighed.

A convenient volumetric method is given in the U. S. Dept. of Commerce, (*infra*).

Chromium.—Chrome-logwood inks are not of such common occurrence as iron-gall inks. When present, chromium can be estimated by fusing the ash with sodium peroxide and sodium carbonate to oxidise the chromium to chromate, dissolving the fused mass in water, boiling to expel hydrogen peroxide, cooling and acidifying with sulphuric acid, adding a measured excess of standard ferrous sulphate solution, and titrating with standard potassium dichromate solution.

Tannins.—It is essential for the permanence of writing that an ink should contain sufficient tannin or gallic acid to form a stable compound with the iron. In the first Prussian regulations of 1888 the source of the tannin was specified, but in the modified regulations of 1912 (*infra*) the gallotannin and gallic acid may be derived from any source, but must answer to the specified tests.

The official German method of estimation is based on extraction of the tannins with ethyl acetate, and titration with iodine. In this method of extraction, devised by Hinrichsen (*Collegium*, 1909, 233, 242), 10 c.c. of the ink are acidified with hydrochloric acid, and shaken out with 50 c.c. ethyl acetate, preferably in Rothe's apparatus (Fig. 1), the extracts united and evaporated *in vacuo*. In using this apparatus the ink is pipetted into the bulb *A*, and 10 c.c. of hydrochloric acid are added through the funnel, followed by 50 c.c. of ethyl acetate. After vigorous shaking, the layers are left to separate, and the aqueous portion is drawn off into the lower bulb,

where it is shaken with more of the solvent, which is introduced through the funnel 4. The aqueous layer is again drawn off through the tap 3, and after removal of the extracts from *A* and *B*, it is returned to the bulb *A*, where the extraction is once more repeated with fresh solvent. Finally the extracts are united, evaporated, and the residue tested. Mitchell's colorimetric method, which is based on a measurement of the pyrogallic groupings (see *Gallic Acid*, III, p. 566) may also be used for estimating the amount of gallotannin and gallic acid in the ethyl acetate extract from the ink.

Another form of extraction vessel, somewhat on the principle of a Soxhlet extractor

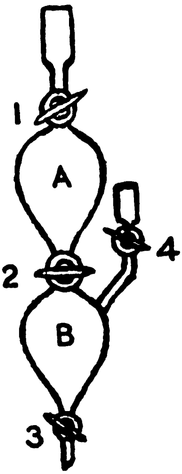

FIG. 1.

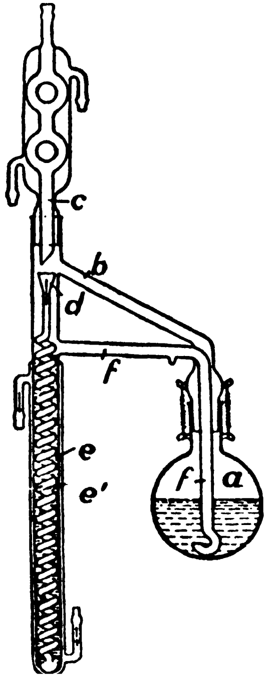

FIG. 2.

has been devised by Kempf (*Mitt. Kgl. Materialsprüfungsamt*, 1913, **21**, 451). (Fig. 2.)

Gums and Dextrin.—An approximate estimation is made by treating 10 c.c. of the ink with 20 c.c. of 95% alcohol, and collecting the precipitate on counterpoised filter papers, which are dried and weighed.

Malagnini distinguishes between gum and dextrin by treating the diluted ink with basic lead acetate and hydrogen sulphide. The filtrate is tested for cane sugar or glucose. For the detection of dextrin a large excess of alcohol is added to a portion of the filtrate;

it gives a white precipitate showing pronounced dextro-rotation in aqueous solution.

Gum arabic is detected by rendering the ink strongly acid with hydrochloric acid and adding a large excess of alcohol. The precipitate (if any) is washed with alcohol and treated with strong hydrochloric acid. A portion of the liquid is then boiled with phloroglucinol, and a violet-rose coloration is produced if gum is present. Another portion is boiled and a few drops of aniline added. In the presence of gum a pink coloration (furfural reaction) is obtained.

Glycerin.—The residue obtained on evaporating the ink is extracted with cold 95% alcohol, the extract evaporated, and the residue heated with potassium bisulphate; fumes of acrolein will be produced in the presence of glycerin. Schiff's test for aldehydes will give a positive result, and a blue coloration will be obtained on treating the extract with a 1% solution of sodium nitroprusside containing a small amount of piperidine.

Phenol and Salicylic Acid.—The total solids from the ink are mixed with sand and extracted with ether, and the extract evaporated. Phenol may be recognised by its odour and by giving a precipitate with bromine water, whilst salicylic acid gives the well-known violet coloration with iron salts.

Dyes.—The diluted ink is acidified with hydrochloric acid and boiled for 15–20 minutes with a thread of wool or cotton. At the same time a parallel test is made with the diluted ink to which ammonia has been added. The threads are washed with hot water, and, if dyed, are boiled with sodium carbonate solution or very dilute acid, and the dyestuffs again fixed on wool or cotton and identified by means of their reactions (see Vol. VI).

Acidity of Ink.—It is essential that the acid in ink should be maintained at a sufficient strength to insure stability (see p. 206). Should the degree of acidity fall below a certain point deposits are formed in the bottle. For this reason it is necessary to test the glass of the bottles to see that they do not yield sufficient alkali to neutralise the acid (Mitchell, *Analyst*, 1921, **46**, 61).

An estimation of the total acidity of writing ink affords a rapid means of checking the work of the ink factory. It may be effected by an electrometric method, or by Mitchell's method of titration after destruction of the dye by means of hydrogen peroxide (*Analyst*, 1921, **46**, 61). For this purpose 5 c.c. of the ink are boiled with

10 c.c. of hydrogen peroxide (10 vol.) under a reflux condenser, until a straw-coloured liquid is obtained. This is cooled, diluted and titrated with N-alkali solution, a blank test being made with 10 c.c. of hydrogen peroxide diluted with 5 c.c. of water. As hæmatoxylin is not bleached by this treatment, the method is modified for logwood inks by boiling 5 c.c. of the ink with 20 c.c. of hydrogen peroxide and 2 c.c. of N-sodium hydroxide solution, and, after 5 minutes, diluting the mixture and titrating the excess of alkali with N-acid, Congo red being used as indicator, since litmus or phenolphthalein are bleached.

Corrosive Acids.—The weak organic acids in ink, such as gallic acid, have no corrosive action on steel pens, but hydrochloric, sulphuric or oxalic acid, which are used as stabilising agents, speedily attack and dissolve the metal.

The proportion of such strong acid may be accurately estimated by Mitchell's method (*Analyst*, 1921, **46**, 61) of distilling 10 c.c. of the diluted ink with an excess of sodium acetate and titrating the acetic acid in the distillate. At least 3 distillations are required to remove the whole of the liberated acetic acid.

The following table gives results thus obtained with typical commercial writing and fountain pen inks, the figures representing the number of c.c. of N-alkali neutralised by the acetic acid liberated by 10 c.c. of the inks:

Ink	Total acid, c.c.	Corrosive acids, c.c.	Weak acids
Logwood ink	1.60	1.76	Nil
Blue black I	1.93	0.30	1.63
Gallic acid ink (acid free)	2.99	0.17	2.82
Fountain pen ink	3.42	1.82	1.60
Record iron-gall ink	5.62	1.55	4.07
Blue-black II	4.06	0.98	3.08
Blue-black, decomposed by alkali of bottle glass	1.92	0.07	1.85

It will be noted that the "total acidity" of the logwood ink as estimated by one method is lower than the corrosive acitity as estimated by distillation.

The small amount of corrosive acid in the acid-free gallic acid ink was derived from the copperas used in the manufacture.

Pen-nib Test.—This is a very crude method, which was first devised by the present writer in 1903, (*Inks and Their Manufacture*)

and has since been officially adopted in the U. S. A. Specifications. It consists in immersing a cleaned and weighed steel pen in a measured quantity of the ink, and estimating the loss in weight after a given period.

Rupert (*J. Ind. Eng. Chem.*, 1923, **15**, 489) avoids some of the obvious drawbacks of this method. Pens of unplated steel of the same size and make are used, and the average of 5 tests on the same ink is taken. The pens are washed with alcohol and with ether, dried and weighed, and one is placed in 10 to 20 c.c. of each of the samples of ink and left for 7 days in loosely covered bottles, after which they are wiped, dried and weighed.

To reproduce, as nearly as possible, working conditions Rupert has devised a mechanical dipping apparatus (*loc. cit.*), by means of which the pen is immersed at regular intervals in the ink and allowed to dry between the immersions. From 5 to 7 days are taken for the test, after which an estimate is formed of the amount of deposition.

Prussians Regulations for Official Tests of Ink[1]

According to the Prussian regulations of May 22, 1912, inks are classified into 1. "documentary" and 2. "writing inks," the latter being subdivided into (*a*) iron-gall inks and (*b*) logwood and dye-stuff inks.

1. "*Documentary ink*" is defined as an iron-gall ink which gives dark writing after eight days' exposure to light and air. It must contain at least 27 grm. of anhydrous gallotannic and gallic acids and 4 grm. of iron (calculated as the metal) per litre. On the other hand, the amount of iron must not exceed 6 grm., so that the ratio of gallotannic and gallic acids to iron must lie within the limits 4.5 : 1 and 6.75 : 1. The ink must not alter for at least fourteen days in the ink-pot, and must flow readily from the pen. The writing done with it must not be sticky immediately after drying, and after eight days it must remain deep black when washed with water and with alcohol (85 and 50 per cent.).

2. *Iron gall "writing inks"* (*a*) must contain at least 18 grm. of gallotannic and gallic acids, and at least 2.6 and not more than 4 grm. of iron per litre (ratio 4.5 : 1 and 6.75 : 1). In other respects they must comply with the requirements of "documentary" inks. Inks of Group B are not officially tested.

Analysis of Inks.—The proportion of gallotannic and gallic acids is estimated by shaking the sample with ethyl acetate and weighing the residue left on evaporating the extract. The residue is regarded as gallotannic and gallic acids when 0.1 grm. thereof absorbs, in the presence of 2 grm. of sodium bicarbonate, at least 0.5 grm. of iodine. If less iodine is consumed the ink is not up to the official requirements.

Estimation of Gallotannic and Gallic Acids.—Ten c.c. of the ink are mixed with 10 c.c. of concentrated hydrochloric acid, and the mixture shaken with successive portions of 50 c.c. of ethyl acetate in Rothe's shaking apparatus, until the aqueous layer gives no reaction for gallotannic or gallic acids after treatment with sodium carbonate and addition of ferric chloride and ferrous sulphate. The ethyl acetate extract is shaken several times with semi-saturated potassium chloride solution (10 c.c. each time) to remove iron salts and then evaporated *in vacuo*, and the residue is taken up with a little water, transferred to a weighed crucible,

[1] Hinrichsen, *Chem. Zeit.*, 1913, xxxvii., 265.

and dried at 105° to 110°, or, preferably, *in vacuo* at about 60° until constant in weight.

In estimating the iodine absorption about 0.1 grm. of the residue is mixed in a stoppered flask with 2 grm. of sodium bicarbonate and 25 to 50 c.c. of a solution of iodine (about 50 grm. per litre), and the flask closed and allowed to stand overnight, after which the unabsorbed iodine is titrated with standard thiosulphate solution. Simultaneously a blank estimation is made.

Iron.—Ten c.c. of the ink are evaporated to dryness, and the residue ignited until free from carbon and then heated with 1 to 2 c.c. of hydrochloric acid (sp. gr. 1.124) until dissolved. The solution is treated with 1 to 2 c.c. of chlorine water and evaporated to dryness, the residue treated with 0.5 c.c. of strong hydrochloric acid to dissolve basic iron salts, and the solution cooled and diluted with 20 c.c. of water. About 1 grm. of potassium iodide is then added, and the separated iodine titrated with N/10 thiosulphate solution, the liquid being meanwhile rapidly warmed to 55° to promote the separation of iodine.

Testing the Writing.—Pieces of standard paper are stretched in a frame inclined at an angle of 45°, and a measured quantity of ink is made to flow down them from a pipette fixed in a special position with regard to the paper. At the same time a parallel test is made upon the same papers with Schluttig and Neumann's standard ink containing 23.4 grm. of gallotannin, 7.7 grm. of crystalline gallic acid, 30.0 grm. of ferrous sulphate, 10 grm. of gum arabic, 2.5 grm. of hydrochloric acid, and 1.0 grm. of phenol. This ink is allowed to stand for at least 4 days at 10° to 15° and decanted from any deposit.

For comparison in the test it is coloured with a suitable dye to match the ink under examination. The paper with the colour stripes of the two inks upon it is exposed to the air for 8 days in diffused daylight, and is then cut horizontally into strips which are immersed in water 50% alcohol and 80% alcohol respectively. No perceptible bleaching of the ink should take place.

U. S. A. Standard for Writing Inks

The U. S. A. Government has adopted the following specification for inks: The ink must be a gallotannate of iron ink, not inferior in any essential quality to one properly prepared according to the following formula: Tannic acid 23.4 grm; gallic acid 7.7 grm.; ferrous sulphate 30 grm.; dilute hydrochloric acid, U. S. P., 25 grm.; phenol 1 grm.; and suitable blue dye, 2.2 grm.; with water to make 1000 c.c. at 15.6°.

Duplicating inks consist of a mixture of pigment (carbon, ferricyanides and ferrocyanides of iron, artificial ultramarine) and oil, such as castor-oil, sulphonated castor oil, rosin oil, cotton-seed oil, sulphonated cotton-seed oil, and mineral oil. Some also contain rosin soap.

The following methods of examining inks are those used by the Bureau of Standards U. S. A. Dept. of Commerce (Circular No. 95, 1920).

ANALYSIS OF WRITING INKS

Total Solids.—Ten grm. are evaporated to dryness, and the residue dried for one hour at 105°.

Ash.—The residue is ignited at a low red heat

Iron.—The ash is dissolved in 25 c.c. of hydrochloric acid, the solution diluted to 400 c.c. and the iron precipitated with ammonium hydroxide and estimated gravimetrically.

Sulphuric Anhydride.—10 grm. of the ink are evaporated in a silica dish, and the residue heated for an hour at 120° and then ignited and cooled, and the dish heated in water for an hour on the water-bath. The solution is filtered, heated for thirty minutes on the water-bath with 5 c.c. of bromine water, acidified with hydrochloric acid, and treated with 10 c.c. of 10% barium chloride solution. The barium sulphate is filtered off after twelve hours.

Tannin.—10 grm. of ink are mixed with 10 c.c. of concentrated hydrochloric acid in Kempf's extraction apparatus, and water added to about 2 inches below

the overflow tube. The bulb is then filled with ethyl acetate and the apparatus shaken until all tannin has been extracted (about two hours). The ethyl acetate extract is washed three times with a semi-saturated solution of potassium chloride to remove any iron salts present, and then evaporated *in vacuo*, the residue taken up with a little water, the solution filtered, if necessary, and evaporated in a weighed dish, and the residue dried at 105° and weighed.

Chromium.—Chromium, which is usually present in logwood inks, is estimated by fusing the ash with 10 grm. of sodium carbonate in an oxidising atmosphere; the fused mass is treated with water, and filtered, the filtrate acidified with acetic acid, heated to boiling, and treated with 10 per cent. barium chloride. In the presence of chromium a yellow precipitate will be obtained. It is collected in a Gooch crucible, ignited, cooled, and weighed as Cr_2O_3.

Specific Gravity.—This is determined at 15.6°, and compared with water at the same temperature.

Streak Tests.—The ink is applied to pieces of all-rag writing paper of good quality, 11 by 5¾ inches, which are clamped on to a glass plate 8 inches wide, inclined at an angle of 45°, pipettes with a capacity of about 0.6 c.c. being used for the purpose. Regular streaks should be obtained, the head being oval, and the remainder of nearly uniform width. In the case of a very fluid ink the head is tapering, and the streak shows rapid contraction.

Resistance to Light and Reagents.—After the ink has become oxidised within the fibres of the paper (which will take from five to eight days) the streaks are cut laterally into four strips. One of these is immersed in water at the ordinary temperature and a second exposed to ultraviolet light (or to direct sunlight), whilst the third and fourth are kept in the dark. Strip No. 4 is subsequently cut into small pieces and tested with various reagents, such as 95% alcohol; 90 parts by volume of water with 10 of ammonium hydroxide; 2% hydrochloric acid; 2% sodium hydroxide solution; and bleaching powder solution containing N/200 available chlorine. The tests are relative, and the results should be compared with those given by the standard ink.

VALUATION OF WRITING INKS

The values to be assigned to the results of a series of tests of different inks are entirely relative, and of course any system may be adopted. For record inks the exposure to sunlight is the most important test, and the following scheme is used in the Bureau of Chemistry, U. S. Department of Agriculture, for rating a standard ink:

Exposure to sunlight	70
Exposure to reagents	10
Keeping quality, penetration, stickiness, fluidity, and action on steel pens	15
Composition	5
Total	100

Other record inks are given values above or below the figures for the standard ink, as the judgment of the analyst may indicate; thus the total for a very good ink may be over 100.

CIRCULAR OF THE BUREAU OF STANDARDS, No. 183

UNITED STATES GOVERNMENT MASTER SPECIFICATION FOR WRITING INK

FEDERAL SPECIFICATIONS BOARD SPECIFICATION No. 164

I. Types.—The writing ink shall be of the following types: (*a*) Blue-black. 1. Fluid, 2. Concentrated, 3. Powder, and 4. Tablets.

II. Material and Workmanship.—Shall be as described under General Requirements.

III. General Requirements.—The writing fluid as received in Type (*a*) 1 or as prepared by diluting or dissolving the material in Types (*a*) 2, 3, and 4 in the amount of water stated on the label, shall be gallotannate of iron ink, not inferior in any essential to one properly prepared according to the following formula, in which all of the ingredients are of the strength and quality prescribed in the United States Pharmacopœia: tannic acid, 11.7; gallic acid crystals, 3.8; ferrous sulphate, 15.0; hydrochloric acid, dilute, U. S. P., 12.5; carbolic acid (phenol), 1.0; soluble blue, Schultz No. 539, 3.5; grms., water to make a volume of 1000 c.c. at 20°.

IV. Detailed Requirements.—Shall be as described under General Requirements.

V. Method of Inspection and Tests.—**1. Method of Taking Samples.**—(*a*) FLUID.—A pint of ink from each delivery of 100 dozen quarts or less shall be taken as a sample.

An original unopened bottle, bearing all of the manufacturer's marks, shall be sent to the testing laboratory when such bottle contains not less than 16 fluid ounces. When the ink is furnished in smaller bottles, enough of them to aggregate at least 16 fluid ounces shall be sent to the laboratory with all marks intact.

(*b*) CONCENTRATED.—Enough of the material to make 16 fluid ounces of full-strength writing fluid shall be sent to the testing laboratory in an original unopened container bearing all of the manufacturer's marks.

(*c*) POWDER.—Enough of the material to make 16 fluid ounces of full-strength writing fluid shall be sent to the testing laboratory in an original unopened container bearing all of the manufacturer's marks.

(*d*) TABLETS.—Enough of the material to make 16 fluid ounces of full-strength writing fluid shall be sent to the testing laboratory in an original unopened container bearing all of the manufacturer's marks.

2. Tests.—The 16-ounce bottle, or the combined contents of the smaller bottles, or the dissolved or diluted solution of ink, shall be allowed to stand undisturbed for 24 hours to permit any sediment to settle. Enough of the clear ink for all of the tests shall then be drawn off in a pipette. The bottle shall then be inverted slowly and the amount and character of any sediment noted.

The sample shall be tested in comparison with a standard ink made according to the formula in III.

Streaks shall be made by allowing measured portions, of about 0.6 c.c. each, of the clear ink to flow freely across a sheet of white bond paper which is pinned to a board or clamped to a pane of glass and held at an inclination of 45°. For better comparison streaks of the standard shall be made on the same sheet as those of the sample.

(*a*) When the streaks are dry, the sheet shall be examined on the front and the reverse sides. The streaks of the sample shall have the same general form as those of the standard. They shall be as uniform in colour when viewed from the front and the back and shall show no more evidence of striking through the paper.

(*b*) The paper shall be cut into inch-wide strips at right angles to the streaks. Some of the strips shall be kept away from light and fumes, and others used for making the following tests, after they have been exposed to diffused daylight for one week.

After a week's exposure to diffused daylight the streaks of the sample shall be as intensely black as those of the standard.

After exposure to direct sunlight for 96 hours, or at a distance of about 10 inches from an arc or ultra-violet light for 48 hours, the streaks of the sample shall show no more evidence of fading than those of the standard.

Strips shall be soaked in water and in 50% alcohol for 24 hours at room temperature. The sample shall show no greater loss of colour than the standard.

Note.—Ethyl alcohol denatured with methanol is suitable for this test.

Strips shall be soaked in bleaching powder solution containing N/200 available chlorine. The effect upon the sample, in comparison with the standard, shall be noted after 15 minutes, 1 hour, and 24 hours at room temperature. The sample shall show no greater loss of colour than the standard.

(*c*) The content of metallic iron shall be estimated in 10 c.c. of the sample by any suitable chemical procedure. The content of metallic iron shall be not less than 0.29 nor more than 0.35 grm. per 100 c.c.

(*d*) Twenty-five c.c. each of the sample and the standard shall be allowed to stand undisturbed in similar colourless glass vessels loosely covered with filter paper to keep out dust. After two weeks' exposure to diffused daylight and air at ordinary room temperature the sample shall be as free from mould and deposit upon its surface and upon the sides and bottom of the container as the standard.

(*e*) The sample shall be no more corrosive to steel pens than the standard. For each sample under test, including the standard, select two new pens from the same box. Clean the pens with alcohol and ether, dry them in an oven at 105°, and weigh each pair together to the nearest milligram. Immerse each pair of pens in 25 c.c. of the ink contained in a small beaker or flask. After 48 hours remove the pens, wash and scrub them with water and a cloth to cleanse them thoroughly, rinse them with alcohol, dry them in an oven, and weigh. If the pens in the sample ink lose more weight than those in the standard, two more tests shall be made. If the loss in one of these tests is greater than the loss in the standard ink, the sample shall be rejected.

VI. Packing and Marking.—No requirements specified.

VII. Additional Information.—The ink is suitable for use with ordinary or fountain pens. It can not be used for permanent records or for making more than one press copy.

VIII. General Specifications.—No requirements specified.

CIRCULAR OF THE BUREAU OF STANDARDS, No. 182

UNITED STATES GOVERNMENT MASTER SPECIFICATION FOR RECORD AND COPYING INK

FEDERAL SPECIFICATIONS BOARD SPECIFICATION No. 163

I. Type.—The record and copying ink shall be of the blue-black type.

II. Material and Workmanship.—Shall be as described under General Requirements.

III. General Requirements.—The ink shall be iron gallotannate ink, not inferior in any essential to one properly prepared according to the following formula, in which all of the ingredients are of the strength and quality prescribed in the United States Pharmacopœia: Tannic acid, 23.4; gallic acid crystals, 7.7; ferrous sulphate, 30.0; hydrochloric acid, dilute, U. S. P., 25.0; carbolic acid (phenol), 1.0; soluble blue, Schultz No. 539, 3.5 grm.; water to make a volume of 1000 c.c. at 20° C.

IV. Detail Requirements.—Shall be as described under General Requirements.

V. Method of Inspection and Tests.—**1. Method of Taking Samples.**—An original unopened bottle, bearing all of the manufacturer's marks, shall be sent to the testing laboratory when such bottle contains not less than 16 fluid ounces.

When the ink is furnished in smaller bottles, enough of them to aggregate at least 16 fluid ounces shall be sent to the laboratory with all marks intact.

The 16-ounce bottle, or the combined contents of the smaller bottles, shall be allowed to stand undisturbed for 24 hours to allow any sediment to settle. Enough of the clear ink for all of the tests shall then be drawn off in a pipette. The bottle shall then be inverted slowly and the amount and character of any sediment noted.

2. Tests.—The sample shall be tested in comparison with a standard ink made according to the above formula.

Streaks shall be made by allowing measured portions, of about 0.6 c.c. each, of the clear ink to flow freely across a sheet of white bond paper which is pinned to a board or clamped to a pane of glass and held at an inclination of 45°. For better comparison streaks of the standard shall be made on the same sheet as those of the sample.

(*a*) When the streaks are dry, the sheet shall be examined on the front and the reverse sides. The streaks of the sample shall have the same general form as those of the standard. They shall be as uniform in colour when viewed from the front and the back and shall show no more evidence of striking through the paper.

(*b*) The paper shall be cut into inch-wide strips at right angles to the streaks. Some of the strips shall be kept away from light and fumes, and others used for making the following tests, after they have been exposed to diffused daylight for one week:

After a week's exposure to diffused daylight the streaks of the sample shall be as intensely black as those of the standard.

After exposure to direct sunlight for 96 hours, or at a distance of about 10 inches from an arc or ultra-violet light for 48 hours, the streaks of the sample shall show no more evidence of fading than those of the standard.

Strips shall be soaked in water and in 50% alcohol for 24 hours at room temperature. The sample shall show no greater loss of colour than the standard.

Note.—Ethyl alcohol denatured with methanol is suitable for this purpose.

Strips shall be soaked in bleaching powder solution containing N/200 available chlorine. The effect upon the sample, in comparison with the standard, shall be noted after 15 minutes, 1 hour and 24 hours at room temperature. The sample shall show no greater loss of colour than the standard.

(*c*) The content of metallic iron shall be estimated in 10 c.c. of the sample by any suitable chemical procedure. The content of metallic iron shall be not less than 0.58 nor more than 0.70 grm. per 100 c.c.

(*d*) Twenty-five c.c. each of the sample and of the standard shall be allowed to stand undisturbed in similar colourless glass vessels loosely covered with filter paper to keep out dust. After two weeks' exposure to diffused daylight and air at ordinary room temperature the sample shall be as free from mould and deposit upon its surface and upon the sides and bottom of the container as the standard.

(*e*) The sample shall be no more corrosive to steel pens than the standard. For each sample under test, including the standard, select two new pens from the same box. Clean the pens with alcohol and ether, dry them in an oven at 105°., and weigh each pair together to the nearest mg. Immerse each pair of pens in 25 c.c. of the ink contained in a small beaker or flask. After 48 hours remove the pens, wash and scrub them with water and a cloth to cleanse them thoroughly, rinse them with alcohol, dry them in an oven, and weigh. If the pens in the sample lose more weight than those in the standard, two more tests shall be made. If the loss in one of these tests is greater than the loss in the standard ink, the sample shall be rejected.

VI. Packing and Marking.—No requirements specified.

VII. Additional Information.—The ink is suitable for writing permanent records. It is not recommended for more than one press copy, nor for use in fountain pens, nor for other ordinary purposes.

VIII. General Specifications.—No requirements specified.

CIRCULAR OF THE BUREAU OF STANDARDS, NO. 184

UNITED STATES GOVERNMENT MASTER SPECIFICATION FOR RED INK

I. Type.—This ink shall be of only one type.

II. Material and Workmanship.—Shall be as described under General Requirements.

III. General Requirements.—The ink shall not be inferior in any essential to one properly prepared according to the following formula: Dissolve 5.5 grm. of crocein scarlet 3B, Schultz No. 227, in 1000 c.c. of distilled or rain water.

IV. Detail Requirements.—Shall be as required under General Requirements.

V. Method of Inspection and Tests.—**1. Method of Taking Samples.**—An original unopened bottle, bearing all of the manufacturer's marks, shall be sent to the testing laboratory when such bottle contains not less than 4 fluid ounces. When the ink is furnished in smaller bottles, enough of them to aggregate at least 4 fluid ounces shall be sent to the testing laboratory unopened and with all marks intact.

2. Tests.—The sample shall be tested in comparison with a standard ink made according to the above formula.

Streaks shall be made by allowing measured portions, of about 0.6 c.c. each, of the ink to flow freely across a sheet of white bond paper which is pinned to a board or clamped to a pane of glass and held at an inclination of 45°. For better comparison, streaks of the standard ink, prepared according to the above formula, shall be made on the same sheet as those of the sample.

(*a*) When the streaks are dry the sheet shall be examined on the front and the reverse sides. The streaks of the sample shall have the same general form as those of the standard. They shall be as uniform in colour when viewed from the front and the back and shall show no more evidence of striking through the paper.

(*b*) The paper shall be cut into inch-wide strips at right angles to the streaks. Some of the strips shall be kept away from light and fumes, and others used for making the following test, after the ink has been allowed to dry for one hour.

After exposure to direct sunlight for 48 hours, or at a distance of about 10 inches from an arc or ultra-violet light for 24 hours, the streaks of the sample shall show no more evidence of fading than those of the standard.

VI. Packing and Marking.—No requirements specified.

VII. Additional Information.—No requirements specified.

VIII. General Specifications.—No requirements specified.

CIRCULAR OF THE BUREAU OF STANDARDS, NO. 187

UNITED STATES GOVERNMENT MASTER SPECIFICATION FOR HECTOGRAPH RIBBONS

FEDERAL SPECIFICATIONS BOARD SPECIFICATION NO. 168

I. Types.—The ribbons shall be of the following types: (*a*) Single colour—1. Purple. (*b*) Two colour—1. Purple and red.

II. Material and Workmanship.—**1. Fabric.**—The cloth shall be made of cotton thoroughly cleaned, combed, and free from waste. It shall be evenly woven and free from an excessive number of avoidable imperfections of manufacture. The weave shall be plain and the yarn single ply.

The thread count shall be not less than 140 threads per inch in warp and filling and the thickness not more than 0.0057 inch. The difference between the warp and filling counts shall not exceed 10 threads.

The edges shall be cut and properly gummed to prevent fraying and shall be without tendency to waviness.

2. **Inking.**—The ribbon shall be coated on one side only with ink suitable for typewriting on paper. The typed writing shall be transferable to a gelatin-glycerin film or to hectograph clay, so that copies on paper can be produced therefrom.

III. General Requirements.—1. **Dimensions.**—The length shall be not less than 9 yards for machines requiring ribbons wider than nine-sixteenths inch and not less than 12 yards for machines requiring ribbons up to and including nine-sixteenths inch, unless shorter ribbons are specifically called for. The width shall be as required for the machine specified in ordering.

IV. Detail Requirements.—Shall be as described above.

V. Method of Inspection and Tests.—1. **Method of Taking Samples.**—One ribbon shall be sent to the testing laboratory in an original unopened container bearing all of the manufacturer's marks.

2. **Tests.**—The fabric shall be examined according to the following methods: Visual examination shall be made of the sample to determine the nature of the edge and the character of the cloth in regard to its freedom from waste and avoidable imperfections of manufacture and to ascertain if the cotton had been combed.

The actual number of threads in 1 inch shall be counted in the filling direction at three different places and the results averaged. The total number of warp threads shall be counted and calculated to a basis of 1 inch.

The width shall be determined by laying the material on a flat surface without tension, then measuring the distance perpendicular to the length between the edges. Three measurements shall be taken at different places in the sample and the results averaged.

The thickness of the de-inked ribbon shall be measured at five different points by means of any suitable gauge and the results averaged.

The ribbon as received shall be tested on a typewriter or suitable automatic testing machine. If the ribbon as received is on a spool that will not fit the machine used for testing, it shall be transferred to a suitable spool.

Place the ribbon in the machine and wind 2 yards of its free end upon the empty spool. This is done so that the ribbon will shift a definite distance while making the following tests.

A sentence about 30 letters in length shall be written 25 times. At the beginning of each line the ribbons shall be reeled back to the starting point.[1] The first line shall be clear and clean, with no blurring of any of the letters. The last line of the 25 shall be distinct and easy to read. The ribbon shall be allowed to stand at rest for one hour, after which another line shall be written. This line shall be as clear and distinct as the third line of the preceding 25.

The writing produced in the above test shall be transferred to a hectograph pad and 25 copies made from it in the usual manner. All of the copies of the first line of writing shall be easily legible.

VI. Packing and Marking.—No requirements specified.

VII. Additional Information.—No requirements specified.

VIII. General Specifications.—No requirements specified.

MODERN CARBON WRITING INKS

The commercial inks used in Egypt, Turkey and other Eastern countries for ordinary writing purposes include inks containing carbon in a fine state of suspension.

[1] This is easily done by making a pencil mark across the ribbon at the point where it leaves the spool holder or at any other convenient fixed point on the machine. The test can not be made by reversing the direction of the ribbon feed at the end of each line, because the ribbon does not travel the same distance in both directions. With some machines it is possible to prevent travel of the ribbon by raising the ribbon-feed pawl. If this can be done, it is preferable to reeling back the ribbon each time.

Analyses of such inks made by Lucas (*Analyst*, 1922, **47**, 9) show that the total solids may range from 8.9 to 16.1%, and the mineral matter from 0.9 to 2.9%. In the best qualities of ink the carbon subsides very slowly, whereas in the cheaper varieties the deposition takes place fairly rapidly.

Artists' drawing inks consist of finely divided carbon suspended in a medium, which is sometimes of a resinous nature, so that an insoluble deposit is left on the paper (*Waterproof Inks*).

Indian and Chinese inks are composed of fine lampblack incorporated into a mass with glue, and then dried, rolled, and stamped into sticks. The best qualities are characterised by their greater covering power and by the fact that when ground up with water the carbon remains in suspension for a much longer time than in the case of the cheaper kinds.

Analyses made by the writer showed the carbon in Indian inks to range from 49.6 to 57.04%, the moisture from 7.2 to 9.93%, and the mineral matter from 3.69 to 4.96%.

The residue left on extracting soluble substances with hot water is practically free from nitrogen, and a test for distinguishing between sepia (made from the pigment of the cuttle fish) and Indian ink has been based by Mitchell on this difference.

CHEMICAL EXAMINATION OF INK IN WRITING

It is frequently a matter of great importance in chemico-legal cases to ascertain the nature of the ink upon a document, to compare it with similar inks upon other documents, and to ascertain the relative ages of two specimens of writing. A minute examination should first be made with a lens magnifying about 10 diameters, and any peculiarities of colour, lustre, shade, etc., noted, and where lines cross each other a note should be made as to which lie uppermost. The examination should then be continued under a microscope, preferably a binocular, which will reveal the inner structure of the ink lines. The examination is often facilitated by moistening the paper with benzene or petroleum spirit, whereby it is rendered semi-transparent. The use of water or alcohol for this purpose is inadmissible.

Differentiation of Inks.—Valuable information may be obtained by comparing the colours of the inks under an Osborn's Comparison microscope. (Osborn *Questioned Documents*, Rochester, U. S. A.

p. 355.) This instrument has two body tubes, each with an objective. These tubes are fitted into a prism box at the top, in which there is a single eye-piece. The effect of the two prisms in the box is to make each half of the field adjacent, when viewed through the eyepiece. Each of the tubes is provided with a sliding shutter, which enables the standard glasses of Lovibond's tintometer to be introduced, and a record taken of the colour of the ink. (See Fig. 3.)

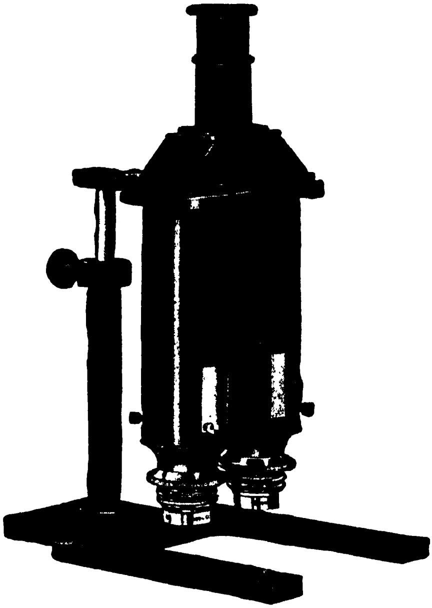

FIG. 3.

Chemical Tests of Inks.—Some inks are affected much more rapidly by reagents than others, though the rate of change depends greatly on the age of the writing. A minute drop of normal oxalic acid (83 grm. per litre), or hydrochloric acid of corresponding strength, should be applied to a part of the ink by means of a capillary pipette, and the action observed under a lens, the reagent being allowed to dry on the paper. Recent writing (1 or 2 days old) in gallic inks free from aniline dyes is changed by 1 application of oxalic acid to a light grey, or by hydrochloric acid to yellow. Older stains resist longer, in proportion to their age, and a deeper colour remains. Logwood ink marks are reddened by oxalic acid, and

alizarine marks become bluish, but aniline inks are unaffected. With hydrochloric acid, logwood ink marks turn reddish or reddish-grey, alizarine marks greenish, and aniline ink marks reddish or brownish-grey. The treatment with acid should be followed by exposure to ammonia vapours, or blotting-paper wet with ammonia may be applied. Thus treated, marks in logwood ink turn dark violet or violet-black.

For the examination of modern blue-black inks Mitchell (*Analyst*, 1898, **33**, 180) found the following reagents to be the most useful: 1. 5% hydrochloric acid. 2. 5% oxalic acid solution. 3. 10% stannous chloride solution. 4. Nascent hydrogen (50% HCl + zinc). 5. Saturated aqueous solution of bromine. 6. Saturated solution of bleaching powder. 7. Titanous chloride (commercial solution). 8. Potassium ferrocyanide (5% containing 1% HCl).

Of these reagents, 1. and 2. act mainly upon the iron tannate and leave the dye. The third and fourth bleach the iron tannate and reduce the dye, and the fifth and sixth oxidise both pigments and cause superficial bleaching. No. 7 reduces both pigments and No. 8 acts mainly upon the iron liberated by the acid. The colorations appearing on the back of the paper are also sometimes characteristic. For further particulars see Mitchell and Hepworth's *Inks* p. 176, and Mitchell's *Documents and Their Scientific Examination*.

Estimation of the Age of Ink in Writing.—Under favourable conditions some idea of the relative ages of ink in writing may be formed. The change of colour may be studied under Osborn's comparison microscope before and after exposure to the air. Any material alteration in the course of a day or too normally indicates that the writing is of fairly recent origin (see *Documents and Their Scientific Examination*, p. 62 *et seq.*).

One of the first to recommend the application of reagents to writing in black ink for the identification of handwriting was W. Thomson (*Chem. News*, **42**, 32).

Robertson and Hoffmann (*Pharm. Centr.*, **33**, 225) proposed a series of tests to be applied to written characters with the following reagents in order to detect forgeries and alterations: (1) 3% solution of oxalic acid in water; (2) 10% solution of citric acid in water; (3) 2% solution of chloride of lime in water; (4) Solution of 1 part stannous chloride in 1 part of hydrochloric acid and 10 parts of water; (5) 15% sulphuric acid solution; (6) 10% hydrochloric acid solution;

(7) 20% nitric acid solution; (8) Saturated solution of sulphur dioxide in water; (9) 4% solution of gold chloride in water; (10) Solution of 1part potassium ferrocyanide in 1 part of hydrochloric acid and 10 parts water; (11) Solution of 1 part sodium thiosulphate in 1 part ammonia and 10 parts water; and (12) 4% solution of sodium hydroxide.

These reagents were applied by means of a quill pen drawn over the characters, and the results obtained with different inks are shown in the following table. The course of the reactions was followed under a power of 100 diameters.

The age of ink marks very greatly affects the rate of their fading when treated with dilute ammonia, the old marks being more refractory. The behaviour of ink marks when treated with solution of bleaching powder is often characteristic, the older writings resisting longer; but unless the reagent be extremely dilute, writings of all ages are removed almost simultaneously (R. Irvine, *J. Soc. Chem. Ind.*, **6**, 807). Hydrogen peroxide acts more slowly than bleaching solution, but gives more definite results.

Mitchell (*Analyst*, 1920, **45**, 247) has shown that the different behaviour of ink in recent and old writing depends upon the gradual change from a soluble iron tannate into first a colloidal tannate and then a resinous tannate, which envelopes the blue dye. Great caution is necessary in drawing conclusions from the results of such tests (*Cf. Documents*, p. 70).

For photographic methods of examining inks (see Mitchell and Hepworth's *Inks* p. 185), and for the methods of examining writing on charred documents see Mitchell (*Analyst*, 1925, **50**, 174).

Restoration of Bleached or Faded Writing.—When the ink in writing has been bleached with a hypochlorite the iron of the ink remains mordanted on the paper, and the mark may be restored by treatment with a dilute solution of gallic acid, gallotannin, or acidified potassium ferrocyanide. The same reagents may be used for restoring writing which has faded from age alone.

When ink marks have been erased or discharged by chemical means, traces of the treatment are often recognisable. After the erasure the spot is sometimes rubbed over with powdered alum or gum sandarac, or coated with gelatin or size. The bleaching agents most likely to have been used are oxalic, citric, or hydrochloric acid, bleaching powder solution or a hypochlorite, or acid sulphite

Reagents	Iron gallotannic ink	Logwood: With potassium chromate	Logwood: With copper sulphate	Nigrosine ink	Vanadium ink	Resorcinol ink
Oxalic acid.	Disappears.	Violet.	Orange-yellow.	Unchanged.	Fades, and runs a little.	Bright red.
Citric acid.	Fades.	Violet.	Orange-yellow.	Runs, with dark blue colour.	Fades and runs.	Disappears.
Hydrochloric acid.	Disappears, but leaves behind a yellow coloration.	Purplish-red.	Blood-red.	Slightly altered.	Fades slightly and runs a little.	Bright pink.
Sulphuric acid.	Disappears.	Red.	Purplish-red.	Unchanged.	Fades a little.	Bright red.
Nitric acid.	Disappears.	Red.	Purplish-red.	Runs a little.	Fades a little.	Bright pink.
Stannous chloride.	Disappears.	Red.	Magenta-red.	Unchanged.	Fades a little.	Disappears.
Sulphurous acid.	Fades.	Greyish-violet.	Red.	Unchanged.	Fades a little and runs	Fades.
Gold chloride.	Fades slightly.	Reddish-brown.	Brown.	Unchanged.	Unchanged.	Runs, with brown colour.
Sodium thiosulphate and ammonia.	Dark red.	Unchanged,	Dark blue.	Dark violet, runs.	Runs very much.	Brown.
Potassium ferrocyanide and hydrochloric acid.	Blue.	Red.	Brick-red.	Unchanged.	Unchanged.	Pink.
Sodium hydroxide.	Dark red.	Brown.	Dark red, runs.	Dark violet, runs.	Dirty brown, runs.	Unchanged.
Chloride of lime.	Disappears.	Disappears.	Disappears, but leaves a yellow coloration.	Brown.	Unchanged.	Brown.

of sodium. Moistened litmus paper will indicate the presence of a free acid, and in some cases treatment with ammonia fumes will restore the colour. The presence of calcium, chlorides, or sulphates in the water in which the paper is soaked will afford some indication of bleaching powder or a sulphite having been used. Potassium ferrocyanide or thiocyanic acid vapour will detect any iron remaining in the paper. Exposure to iodine vapour often affords evidence of chemical treatment, and other methods of examination readily suggest themselves.

When characters have been removed by other than chemical means, proof may be obtained by means of a photograph taken by transmitted light, or by exposing the paper to iodine vapour. The latter process is especially useful in cases where, for the removal of the writing, the paper has been moistened; these places become blue, the others brown. When the removal has been effected by chemical means, in most cases by oxalic acid, chloride of lime or sulphur dioxide, the suspected places are treated with a solution of sulphur dioxide in water, then with 3% solution of hydrogen peroxide, and finally with dilute ammonia. After the evaporation of the excess of ammonia, good results may be obtained with tannin, which darkens the characters.

TYPE WRITING INKS

The early inks used for impregnating the ribbons or pads of typewriters consisted of a solution of an aniline dye (methyl violet, methylene blue) in water or spirit thickened with glycerin. Inks of this type are still in use, although they have been to a large extent replaced by inks approaching more nearly to the type of printing inks.

Black pigments, such as lamp black and carbon black, and mineral pigments such as Prussian blue or antimony cinnabar, are finely ground in mills with the liquid medium, which sometimes consists of a mixture of heavy mineral oil, oleic acid and paraffin wax, whilst a small amount of aniline dye such as nigrosine or indulin blue is also added. Colour lakes are also used as ingredients, and the tint may be corrected by the addition of zinc white.

TYPEWRITER RIBBONS

The methods of testing typewriter ribbons given here are those which have been used in the Bureau of Chemistry, U. S. Department of Agriculture (*Bulletin* 109, Revised).

The important things to be ascertained in a typewriter ribbon are length, width, typefilling, character of record (and of copy if it is a copy ribbon), life of ribbon, and resistance of record and copy to reagents and sunlight.

Length and width are frequently specified and these should be recorded, but the rating should be based entirely on quality.

Typefilling.—Take a sufficiently long section of the ribbon on a suitable typewriter (for wide ribbon use a Remington No. 6 and for a narrow ribbon an Underwood No. 5). Carefully clean the small letter *e*, and using the standard paper used for ink testing make records of the small letter *e*, striking the key with as uniform a stroke as possible. Continue writing the letter *e* until the loop fills so as to demand cleaning the type, or until 8000 impressions have been made. Ribbons which give 8000 clear impressions are given a maximum rating on typefilling, though the test may be carried as much further as may be desired.[1]

Character of record may be determined by examining the sheets from the typefilling test. Note colour, clearness, whether the ribbon appears over- or under-inked, and the tendency to smear. If the ribbon is a copy ribbon, make a press copy of the record and make the same observations on the copy and copied original as on the original record.

Life of Ribbon.—For this test a special machine is used. The machine has ten type, the letters A, E, I, O, U, both capitals and small letters. Each type is mounted on the end of a plunger weighing 60 grm. The plunger is raised by a cam and a uniform blow is obtained by allowing it to fall freely for 1.8 cm. The plungers fall one after another in a manner similar to that on a type-writing machine, except that each type strikes a different part of the ribbon from the other type; but the successive blows from the same type fall on the same spot on the ribbon. The cams are operated by a geared mechanism which is operated from an electric motor connected to the testing machine proper by a belt. When a complete line has been struck, the platen roll is moved forward one space

[1] It is exceedingly difficult for an operator to use a uniform stroke in making this test. This makes the comparison rather difficult. In order to avoid this personal equation, P. H. Walker has devised a machine for automatically striking the letter "*e*." This machine is attached to a standard typewriter, the motive power being a low speed electric motor, which, by a suitable system of gearing, operates a hammer which strikes the letter "*e*" a perfectly uniform stroke. When the carriage has proceeded to the end of its course, a blank portion of one of the gear wheels causes the operation of the hammer to cease, and at the same time a lever operates a pulley which draws the carriage back and shifts the roll so that the operation is continuous.

automatically. The ribbon is clamped firmly at one end and the other end passes over a bar and has clamped to it a weight of 45 grm., thus insuring a uniform tension on all ribbons tested. The paper, in strips 14 cm. wide and 41 cm. long ($5\frac{1}{2} \times 16$ inches), or fed from a long roll works under the ribbon as in a typewriter. The life of the ribbon is measured by the number of lines of writing which can be made before one or more holes wear in the ribbon, or before one or more type fail to make a clear impression.

Resistance to Sunlight and Reagents.—Cut up the sheets used in determining typefilling and character of record, and subject original, copy, and copied original to the same tests as to resistance to sunlight and reagents that are used on writing inks. Expose to sunlight for at least 14 days, or longer if possible.

Rating.—Length and width are recorded, but are not included in the rating which is based on quality only, the ribbons being marked on a scale of 100.

Typefilling is given a maximum rating of 5 and is marked as follows:

1000 or fewer clear impressions of the small letter *e*	1
1000 to 3000 clear impressions of the small letter *e*	2
3000 to 5000 clear impressions of the small letter *e*	3
5000 to 8000 clear impressions of the small letter *e*	4
8000 or more clear impressions of the small letter *e*	5
Character of record is given a maximum rating of	20
Life of ribbon is given a maximum rating of	10
Resistance to reagents is given a maximum rating of	20
Resistance to sunlight is given a maximum rating of	45

CIRCULAR OF THE BUREAU OF STANDARDS, No. 186

UNITED STATES GOVERNMENT MASTER SPECIFICATION FOR TYPEWRITER RIBBONS

FEDERAL SPECIFICATIONS BOARD, SPECIFICATION No. 167

This specification was officially promulgated by the Federal Specifications Board on June 30, 1924, for the use of the Departments and Independent Establishments of the Government in the purchase of typewriter ribbons.

I. Types.—The typewriter ribbons shall be of the following types: (*a*) Single colour—1. Black record, 2. black copying blue, and 3. blue record.

(*b*) Two colour—1. Red and black record and 2. red and black copying.

II. Material and Workmanship:—**1. Fabric.**—The cloth shall be made of cotton thoroughly cleaned, combed, and free from waste. It shall be evenly woven and free from an excessive number of avoidable imperfections of manufacture. The weave shall be plain and the yarn single ply.

The thread count shall be not less than 140 threads per inch of warp and filling, and the thickness not more than 0.0057 inch. The difference between the warp and the filling counts shall not exceed 10 threads.

The edges shall be cut and properly gummed to prevent fraying and shall be without tendency to waviness.

2. Inking.—All ribbons shall be non-type filling, and all colour used in the ink shall be as permanent as possible. The ink shall be uniformly applied, and different weights of inking shall be furnished as required for elite or pica type to produce satisfactory work on the different stroke machines. Copying ribbons shall give clear impressions and satisfactory press copies.

III. General Requirements.—1. Dimensions.—The length shall be not less than 9 yards for machines requiring ribbons wider than nine-sixteenths inch, and not less than 12 yards for machines requiring ribbons up to and including nine-sixteenths inch in width, unless shorter ribbons are specifically called for. The width shall be as required for the machine specified in ordering.

IV. Detail Requirements.—Shall be as described above.

V. Method of Inspection and Tests.—1. Method of Taking Samples.—One ribbon from each delivery of 200 dozen or less shall be sent to the testing laboratory in an original unopened container bearing all of the manufacturer's marks.

2. Tests.—The fabric shall be examined according to the following methods: Visual examination shall be made of the sample to determine the nature of the edge and the character of the cloth in regard to its freedom from waste and avoidable imperfections of manufacture and to ascertain if the cotton had been combed.

The actual number of threads in 1 inch shall be counted in the filling direction at three different places and the results averaged. The total number of warp threads shall be counted and calculated to a basis of 1 inch.

The width shall be determined by laying the material on a flat surface without tension, then measuring the distance perpendicular to the length between edges. Three measurements shall be taken at different places in the sample and the results averaged.

The thickness of the de-inked ribbon shall be measured at five different points by means of any suitable gauge and the results averaged.

The ribbon as received shall be tested on a typewriter or suitable automatic testing machine. If the ribbon as received is on a spool that will not fit the machine used for testing it, it shall be transferred to a suitable spool.

Place the ribbon in the machine and wind 2 yards of its free end upon the empty spool. This is done so that the ribbon will shift a definite distance while making the following tests:

A sentence about 30 letters in length shall be written 25 times. At the beginning of each line the ribbon shall be reeled back to the starting point.[1] The first line shall be clear and clean, with no blurring of any of the letters. The last line of the 25 shall be distinct and easy to read. The ribbon shall be allowed to stand at rest for one hour, after which another line shall be written. This line shall be as clear and distinct as the third line of the preceding 25.

The writing produced by this test shall be half covered with black paper and exposed to direct sunlight, or at a distance of about 10 inches from an arc or ultra-violet light. Writing made with black record ribbons shall show no appreciable fading after exposure to direct sunlight for 96 hours or to arc or ultra-violet light for 48 hours. Writing made with coloured ribbons, or press copies therefrom, after exposure in a similar way to direct sunlight for 48 hours, or to arc or ultra-violet light for 24 hours, shall still be easily legible.

The small letter "e" shall be thoroughly cleaned and 800 impressions made with it with the normal feed of the ribbon. There shall be no evidence of filling of the type.

VI. Packing and Marking.—No requirements specified.

VII. Additional Information.—Only the ribbons of Type (*a*) 1 shall be used for writing permanent records.

VIII. General Specifications.—No requirements specified.

[1] This is easily done by making a pencil mark across the ribbon at the point where it leaves the spool-holder or at any other convenient fixed point on the machine. The test can not be made by reversing the direction of the ribbon feed at the end of each line, because the ribbon does not travel the same distance in both directions. With some machines it is possible to prevent travel of the ribbon by raising the ribbon-feed pawl. If this can be done, it is preferable to reeling back the ribbon each time.

CIRCULAR OF THE BUREAU OF STANDARDS, No. 188

UNITED STATES GOVERNMENT MASTER SPECIFICATION FOR RIBBONS FOR COMPUTING AND RECORDING MACHINES

FEDERAL SPECIFICATIONS BOARD SPECIFICATION NO. 169

This specification was officially promulgated by the Federal Specifications Board on June 30, 1924, for the use of the Departments and Independent Establishments of the Government in the purchase of ribbons for computing and recording machines.

I. Types.—The ribbons shall be of the following types: (*a*) Single colour: 1. Record—Black, blue, purple, and red. 2. Copying—Black, blue, purple, and red. (*b*) Two colour and three colour: The proportionate parts of two or three of the following colours shall be specified in the order: Black, blue, purple, and red.

II. Material and Workmanship.—**1. Fabric.**—The cloth shall be made of cotton thoroughly cleaned, combed, and free from waste. It shall be evenly woven and free from an excessive number of avoidable imperfections of manufacture. The weave shall be plain and the yarn single ply.

The thread count shall be not less than 140 threads per inch in warp and filling and the thickness not more than 0.0057 inch. The difference between the warp and filling counts shall not exceed 10 threads.

The edges shall be either cut or selvage, according to the machine on which the ribbon is to be used. Cut edges shall be even and properly gummed to prevent fraying and shall be without tendency to waviness. The material shall have a breaking strength of not less than 40 pounds per inch of width.

2. Inking.—Ribbons shall be evenly and heavily inked, but free from an excess of ink which would tend to fill the type. Ink other than black need not be of a permanent nature. The ribbons shall give clear impressions from the start.

III. General Requirements.—**1. Dimensions.**—Ribbons shall be furnished of the length and width required for the machine on which they are to be used.

IV. Detail Requirements.—Shall be as described above.

V. Methods of Inspection and Tests.—**1. Method of Taking Samples.**—One ribbon shall be sent to the testing laboratory in an original unopened container bearing all of the manufacturer's marks.

2. Tests.—The fabric shall be examined according to the following methods: Visual examination shall be made of the sample to determine the nature of the edge and the character of the cloth in regard to its freedom from waste and avoidable imperfections of manufacture and to ascertain if the cotton had been combed.

The actual number of threads in 1 inch shall be counted in the warp and filling directions at three different places and the results averaged. If the ribbon is narrower than 1 inch, the total number of warp threads shall be counted and calculated to a basis of inch.

The width shall be determined by laying the material on a flat surface without tension, then measuring the distance perpendicular to the length between the edges. Three measurements shall be taken at different places in the sample and the results averaged.

The thickness of the de-inked ribbon shall be measured at five different points by means of any suitable gauge and the results averaged.

Three test specimens approximately 6 inches long shall be cut, one from each of the ends and one from the middle of the ribbon. If the ribbon is wider than 1 inch, each specimen shall be raveled to exactly 1 inch in width by taking from each side approximately the same number of threads. If the ribbon is narrower than 1 inch, the results of the breaking tests, shall be calculated to a basis of 1-inch width.

The testing machine used shall be of the inclination-balance type. The capacity of the machine shall be such that the arm will not go beyond a maximum

angle of 45° in breaking the strips (machines of less capacity tend to give low results). The lower or pulling jaw shall travel at a uniform rate of 12 inches per minute under no load. The distance between the jaws shall be 3 inches at the start of the test. The width of the jaws shall be 1½ inches or more. The jaws shall have a smooth and flat surface, with edges slightly rounded to prevent cutting.

The results of the breaking-strength tests shall be averaged. If a specimen slips in the jaws, breaks in the jaws, breaks at the edge of the jaw, or if for any reason due to faulty operation one of the results falls markedly below the general average, that result shall be diregarded, another specimen shall be taken from the adjacent part of the ribbon, and the result of this break shall be included in the average.

The ribbon as received shall be tested on the machine for which it is intended, on a typewriter, or on a suitable automatic testing machine. If the ribbon is on a spool that will not fit the machine used for testing, it shall be transferred to a suitable spool.

Place the ribbon in the machine and wind 2 yards of its free end upon the empty spool. This is done so that the ribbon will shift a definite distance while making the following tests: The figures 0 to 9 shall be written 50 times over the same length of ribbon. At the beginning of each line the ribbon shall be reeled back to the starting point.[1] The first line shall be clear and clean, with no blurring of any of the letters. The last line of the 50 shall be distinct and easy to read. The ribbon shall be allowed to stand at rest for one hour, after which another line shall be written. This line shall be as clear and distinct as the fifth line of the preceding 50.

The figure 8 shall be thoroughly cleaned and 200 impressions made with it with the normal feed of the ribbon. There shall be no evidence of filling the type.

In testing copying ribbons, in addition to the above tests, press copies shall be made in the usual way. The copies shall be of good colour, sharp, and easily legible.

VI. Packing and Marking.—No requirements specified.

VII. Additional Information.—Only the black record ribbons, Type (*a*) 1 black, shall be used for writing permanent records.

VIII. General Specifications.—No requirements specified.

CARBON PAPERS

The papers used for duplicating pen or pencil writing or type writing are of a special texture, only rag paper being suitable for pen carbons.

The old carbon papers in manifold books consisted essentially of a coating of lampblack, wax and oil in varying proportions. They were greasy to the touch and readily smudged. Modern "carbons" for typewriters or for duplicating pen writing are made by coating the paper, which must be of a special texture, with a suitable mixture of pigment and medium. The pigments used include lampblack, Prussian blue and organic red, blue and green lakes, the medium is

[1] This is easily done by making a pencil mark across the ribbon at the point where it leaves the spool holder or at any other convenient fixed point on the machine. The test can not be made by reversing the direction of the ribbon feed at the end of each line, because the ribbon does not travel the same distance in both directions. With some machines it is possible to prevent travel of the ribbon by raising the ribbon-feed pawl. If this can be done, it is preferable to reeling back the ribbon each time.

composed of mixtures of waxes and oils chosen to give a composition of the required m. p. and consistence. Commercial carbons may contain several of the following ingredients: Carnauba wax, montan wax, paraffin wax, vaseline, castor oil, oleic acid, stearic acid, pitch, etc.

Analysis of the composition is of less value than the behaviour of the paper in practice, but some idea of the nature of the composition on a paper may be gained by treating the mass with successive solvents, such as dilute alcohol, acetic acid, petroleum spirit, etc., and examining the different extracts.

Practical Tests.—The practical testing of carbon paper consists in making a carbon copy on a good quality of medium weight writing paper, such as is used in testing typewriter ribbons, on the special machine used for testing the life of ribbons. The record is examined in the same manner as the ribbon record and is rated as follows:

Character of record is given a maximum rating of	35
Resistance to reagents is given a maximum rating of	20
Resistance to sunlight is given a maximum rating of	45
Total maximum	100

No test need generally be made of the life of a carbon paper, since when struck in the same place all papers are very soon exhausted, no very pronounced difference in this respect having been observed. Of course, in actual use a number of impressions can be made, since the type seldom strike in the same place.

Cancelling Inks Having an Oil Base[1]

The following methods have been devised for the purpose of ascertaining the suitability of cancelling inks for the use of the Post-Office Department, U. S. A. Many of these methods will be found of assistance in passing judgment upon the quality of stamping inks for miscellaneous uses.

It is important that the ink used by Post-Office Departments for post-marking possess in the highest possible degree certain properties. The ink, first of all, must produce an indelible cancellation; that is, it must be relatively indelible as compared with the ink used for printing the postage stamps. The postmark made with the ink must dry quickly in order that the mails may be handled immediately without

[1] *Bulletin* 109, *Revised, Bureau of Chemistry, U. S. Department of Agriculture*, also Circular No. 95, *Bureau of Standards, U. S. Dept. of Commerce*, 1920.

any blurring or smearing of the postmark. Both this property and the property of indelibility involve the question of the rate at which the ink penetrates or is absorbed by the fibre of the paper. A satisfactory ink does not harden or form a crust on the ink pad on exposure to the air. There must be no deposition of solid matter on the bottom of the vessel in which the ink is stored, and the pigments, on which the indelibility of the ink depends, if insoluble, must not settle out in such a way as to make it possible to pour off from the top of the container a portion of the ink which contains little or none of the insoluble pigment or pigments. The following methods have been found of value for the purpose of ascertaining the quality of a given sample of ink as well as the appropriateness of certain materials used for the manufacture of cancelling inks.

1. **Preparation and Care of the Sample.**—Since cancelling inks contain more or less insoluble and volatile matter, special attention must be given to the preparation and care of the sample. It must be carefully mixed by shaking before each portion is removed for analysis, and the container must be left open no more than is absolutely necessary for the removal of portions of the ink.

2. **Estimation of Matter Volatile at Ordinary Temperatures.**—Place a carefully weighed quantity (between 5 and 5.2 grm.) of the ink in a flat-bottomed aluminium dish 102 mm. (4 inches) in diameter. Distribute the ink completely over the surface of the bottom of the dish by gently tilting the same. This quantity of ink should be sufficient to cover the bottom of the dish. Place the dish on a horizontal shelf or table where air will have free access to it, and where it will be screened in such a way that no dust can fall into it.

Re-weigh the dish at the end of 18 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 14 days, 21 days, and 28 days. Calculate the total percentage loss of weight at the end of each period of drying. The loss of weight should be gradual and should not exceed 15% during the first 7 days, nor 25% during 28 days. This test shows the absence of highly volatile ingredients and the absence of an excess of matter volatile at the ordinary room temperature. The constituents of a cancelling ink should be such that the volatile matter will not exceed the above limits when the ink is exposed under the conditions named to a summer temperature of 26.6° (80° F.) and upwards.

3. **Estimation of Relative Penetrating Power.**—(*a*) **Apparatus.**

(*a*) Homæopathic shell vials about 6 cm. long and 2 to 2.5 cm. in diameter.

(*b*) Strips of white blotting paper, which for a given series of estimations should be cut from the same sheet and of exactly the same estimations. A convenient size is 12 mm. wide and 25 cm. long.

(*c*) A pair of dividers with arms 15 cm. long or longer.

(*d*) A millimetre rule.

(*b*) Estimation

Place approximately 5 c.c. of the ink or other material to be tested in one of the "shell vials" described, and if several samples are to be tested, arrange the vials in a row. Place 5 c.c. portions of distilled water in each of 2 of the vials, and put one of the vials containing water at each end of the row of vials containing samples to be tested. Proceeding from left to right, insert a strip of blotting-paper in each of the vials, recording the exact time the paper was placed in each vial. The blotting-paper should maintain a nearly upright position. The liquids gradually ascend the strips by capillarity; the strips, however, should be in such a position that the liquid does not ascend by capillarity between the edges of the strips and the sides of the vials.

At the expiration of exactly 15 minutes from the time each strip is inserted in the vial, measure the height to which the liquid has ascended the strip of paper by means of a pair of dividers and record the distance in millimetres. Make a second set of readings at the end of 45 minutes.

After all the measurements have been recorded reduce the results to the terms of the penetrating power of distilled water, taking the penetrating power of distilled water as 100. This is done by dividing each result by the average of the results obtained for the distilled water contained in the vials and multiplying the quotient by 100. 10 samples may conveniently be tested at one time by working as described. The method gives good comparative results, and has been applied not only to cancelling and other stamping inks, but also to the liquids used for the manufacture of these inks.

In observing the penetrating power of a given sample of ink it is important to remember that the value of a cancelling or stamping ink depends upon its power to penetrate the paper during the first minute or fraction of a minute following its application to the paper. It is

well, however, to keep the tests under observation for several hours, as information can thus be obtained in regard to the extent to which the colouring matter contained in the ink follows the liquid base of the ink as it passes through the paper. In some cases the colouring matters keep pace with the liquid portions of the ink; in others an uncoloured band at the top of the portion of the paper which is wet with the ink shows that the colouring matter does not proceed through the paper as rapidly as the base of the ink. This may or may not be an undesirable result. If the colourless band is due to a difference in the rate of penetration, it is undesirable. If it shows that the dye contained in the ink has an affinity for the fibres of the paper, it is evidence of a valuable quality.

Additional information can be obtained from the penetration test by removing the strips of paper from the vials, cutting off the part of the paper which has actually been immersed in the ink, and treating the upper part successively with petroleum spirit, ether, alcohol, and other solvents for removal of the constituents of the ink soluble in these liquids. The extent to which the dye or dyes contained in the ink resist the action of these solvents and the extent to which the lampblack has passed up the strip of blotting paper are indices of the quality of the ink.

An examination of the strips with the microscope will give valuable information regarding the rise of carbon in the paper and the affinity of the dye for the fibres of the paper. With many inks the carbon will not rise above the surface of the liquid, while with others it penetrates the paper to the same height as the dye. With this class of inks it is important that the base of the ink have the power to carry the carbon well into the fibres of the paper.

To make the estimation allow the strips to remain in position until the next day, remove, dry between blotters, and examine for a rise of dye or carbon. If either dye or carbon rises as far, or nearly as far, as the base of the ink the rise is pronounced "satisfactory." Less than this is not acceptable except in the case of glycerin inks, which rarely show any rise of carbon. A small amount of rise in the latter inks must be accepted as satisfactory. To determine whether the material that has risen is carbon or simply dye, pick off particles of the paper and adhering ink at intervals on the strip and mount on slides with water or alcohol. Examine with the microscope, using low power; note size of the carbon grains. The grains adhere to the

outside of the paper fibres in clots, as a rule. It is often difficult to distinguish the carbon grains.

4. Sedimentation Test.—(*a*) **Apparatus.**—1. Glass-stoppered cylinders, graduated for 200 c.c. and fractions thereof, the distance between the bottom and the 200 c.c. mark being 25 cm. (10 inches). If unobtainable, other cylinders may be substituted, marks being placed at distances 25 cm. (10 inches) and 16 mm. (10/16 inch) from the bottom.

2. A pair of dividers with arms 15 cm. long or longer.

3. A millimetre rule.

4. Pipettes made from straight tubing (7 mm.), at least 30 cm. in length and having a cpaacity of from 10 to 15 c.c.

(*b*) **Estimation.**—By means of the special pipette introduce carefully, drop by drop, into one of the 200 c.c. cylinders, exactly 16 mm. depth of the ink to be tested. The ink should be previously tested to determine a proper solvent for both base and dye. Alcohol is generally the solvent to use for rosin inks. It may be necessary to use other solvents, such as petroleum spirit (gasoline) (b. p., 50° to 60°), ether, benzene, etc. Dissolve the ink in the cylinder in the appropriate solvent and dilute up to the 200 c.c. mark, stopper, and shake thoroughly. Allow the cylinder to stand, and record from time to time, by using the dividers and millimetre rule, the height of the top of the layer of sediment which collects in the bottom of the cylinder, expressing results in millimetres. During the first hour observations should be made at intervals of 15 minutes; later, each hour for several hours successively, and then twice daily for a week to 10 days.

After the settling of the top of the layer of sediment has entirely ceased, the height of the sediment should equal or exceed 16 mm., the amount of ink taken for the test. The rate of sedimentation is an index of the state of division of the carbon, some inks showing no appreciable layer at the expiration of a 10-day test.

In the case of some inks the supernatant liquid above the sediment is of such a dark colour that there is difficulty in locating the top of the sediment, even when the cylinder is inspected by light reflected at various angles. In this event, the use of a dark room with a light placed so as to give a strong ray through a small aperture will locate the top of the layer of sediment in all cases except when the ink contains a very large percentage of a dense dye.

Frequently the layer may be located by holding an incandescent electric light at the back of the cylinder and noting where the carbon filament cannot be seen. The test is somewhat crude and only approximate, but it serves to give an idea of the fineness and amount of carbon, and, as a rule, agrees fairly well with the carbon determinations.

5. **Lampblack.**—Charge a porcelain Gooch crucible with asbestos, using a felt about ¼ inch thick. After washing the felt thoroughly with water to remove fine particles, finally wash with alcohol and ether, dry, and weigh. Weigh out about 5 grm. of ink in a small beaker, dilute with a suitable solvent (alcohol is used in case of rosin-oil inks), transfer to a Gooch crucible, and wash until all oil and soluble colour is removed. Finally, wash with alcohol and ether, dry, and weigh.

No one solvent can be used for all oil base inks. The analyst should determine the most suitable solvent by tests on separate portions of the ink in question. Filtering on a Gooch crucible is sometimes very tedious, and better results may frequently be obtained by mixing a weighed quantity of the ink in a suitable tube with the solvent, and settling out the pigment by whirling in a centrifugal machine. By pouring off the clear solvent and repeating the extractions, the solid pigment may be finally collected in the tube in which it is weighed after evaporating off the small amount of solvent which cannot be decanted.

6. **Ash.**—The lampblacks prepared for the manufacture of cancelling ink yield less than 0.5% of ash when burned, and the coal tar dyes employed should contain no mineral matter other than that which is an essential part of the molecules of the substances to which the tinctorial power of these dyes is due.

For the estimation of the ash, place 2 to 3 grm. of the ink in a porcelain dish, which must be of such size as to avoid loss of ink due to the foaming which is likely to attend the beginning of the incineration. Heat the dishes thus charged in a muffle at a low red heat, until all organic matter and uncombined carbon have been burned. Cool and weigh.

If an excessive percentage of ash is found, the percentage of mineral matter contained in the alcoholic extract should be estimated by incineration of the residue obtained after evaporation of this

extract. If either the total ash or the ash of the alcoholic extract is high, a qualitative examination should be made.

7. Resistance of Pigments and Dyes to Light and Reagents. It is necessary in the case of cancelling inks, and important, if not necessary, in the case of many stamping inks, that the pigments and dyes employed in their manufacture be as resistant as possible to means which may be employed for the erasure of marks made by them on paper. Under this heading may be mentioned also the importance of dyes which possess considerable affinity for vegetable fibres. It is not practicable to enumerate the agents which should be employed in experiments to ascertain the resistance of a given dye to erasure, as light, heat, and all of the solvents and reagents known to the chemist are available for the use of persons who might desire them for use in assisting them in making fraudulent erasures.

For the purpose of cancelling postage stamps, it is necessary that the cancelling marks be substantially indelible, because the inks used in printing many of the stamps are very resistant. Stamping inks used for other purposes, however, do not demand absolute indelibility.

In making the tests use several layers of blotting paper as a pad, pour on this a small quantity of the ink and distribute carefully; see that all excess has been absorbed by the pad before using the stamp. Make a sufficient number of impressions at one time to suffice for all tests and leave some in reserve. Having made the impressions, arrange them in groups according to the colour and kind of ink, and rank them according to the following scheme:

1. Of highest rank.
2. Very good, but not of the best.
3. Good.
4. Fair.
5. Poor.

Having exposed the impressions to the various reagents, as described hereinafter, each sample is again rated according to the effect of the reagents, as follows:

1. Unaffected.
2. Slightly affected.
3. Much affected.
4. Almost effaced.
5. Effaced.

The wet reagents used are pure water, water with the addition of 10% of strong ammonia (sp. gr. 0.90), pure alcohol (95%), alcohol with the addition of 10% of strong ammonium hydroxide, 2% hydrochloric acid, and N/200 bleaching powder. Expose each impression in a small Erlenmeyer flask to about 50 c.c. of the reagent for 24 hours, noting its appearance at the end of 15 minutes, 1 hour, and 24 hours. Then rinse dry and rate.

For the sunlight test expose impressions under glass for 10 days to direct sun, rating at the end of the third, seventh, and tenth days. The tests with reagents are considered of less use than the other tests, and are not always applied.

RUBBER-STAMP INKS

1. **Preparation and Care of Sample.**—The precautions given in regard to the care of samples of inks made with an oil base should be observed.

2. **Change of Weight on Exposure to Air.**—This estimation should be conducted in the manner described for the estimation of volatile matter in inks made with an oil base. Rubber-stamp inks, however, gain or lose in weight according to the constituents used in their manufacture and according to atmospheric conditions. A rubber-stamp ink should not, however, undergo very much greater changes in weight when exposed to the air under given conditions than diluted glycerin containing 75% of glycerin and 25% of water by volume.

3. **Penetrating Power.**—This test should be conducted in the manner described for inks made with an oil base.

4. **Sedimentation Test.**—This test should be conducted as described for inks made with an oil base, with the exception that the portions of ink should be diluted with water instead of with organic solvents.

5. **Estimation of Lampblack and Other Constituents.**—A scheme of analysis similar to that described for inks made with an oil base should be employed. Some experiments will be necessary in most cases to ascertain the proper solvent to be used in the case of each sample of ink to be examined. Alcohol, however, will generally be found to be satisfactory for rubber-stamp inks. This test is difficult and can not be carried out without a slight loss. To make the loss

as small as possible use a very thick felt of asbestos and make the filtration continuous—never allow the Gooch crucible to run empty; if it does, it is generally better to begin all over again.

6. Resistance to Light and Reagents.—The remarks made in regard to the investigation of the resistance of cancellations made with oil inks apply in general to cancelling and other inks for use with rubber stamps.

Materials Used for the Manufacture of Cancelling and Other Stamping Inks

1. Volatility and Penetrating Power.—The methods which have been described above will be found useful in determining the suitability of liquids for use as bases or constituents of bases of cancelling and other stamping inks.

2. Sedimentation Test.—A modification of the sedimentation test described may be employed with good results for the purpose of ascertaining the suitability of lampblack and other pigments for use in the manufacture of cancelling and other stamping inks. The results, of course, are mainly of value for purposes of comparison.

The conditions of the test may be modified to suit the purposes of the investigation and the character of the materials to be compared. The writer has obtained good results in the comparison of lampblanks and other blacks rich in uncombined carbon by the following method, which was so planned that the results might be applied to stamping inks made with either a water-soluble base or an oil base:

Mix 0.5 grm. of the black to be tested in a mortar with dilute glycerin (87.5 c.c. of glycerin diluted with water to 1 litre). Rinse the mixture into a 100 c.c. Nessler cylinder and dilute to the 100 c.c. mark, using the same dilute glycerin. After having prepared a series of tubes, each containing a portion of one of the blacks to be tested, close each tube with a cork and shake thoroughly each tube successively, performing the operation as quickly as possible in order that the time of settling may be approximately the same in the case of each sample. Allow the cylinders to stand at rest in a place free from jar, and record from time to time the height of the sediment formed by the deposition of the blacks. When submitted to this test, a black which is suitable for the manufacture of a cancelling or stamping ink should occupy a volume of not less than 25 c.c. when the sediment has stopped settling.

3. **Ash, Etc.**—Blacks, dyes, and other substances used for the manufacture of cancelling and other stamping inks should be carefully examined to insure the absence of considerable percentages of substances which are not essential to the production of an ink of good quality. It can generally be assumed that the presence of considerable quantities of any substance which does not actually contribute to the desirable qualities of the ink will detract therefrom. Black pigments rich in carbon of high sp. gr., due to the presence of a large percentage of ash, are highly unsuitable for the manufacture of stamping inks. Only the concentrated brands of coal tar dyes should be used, unless the substances with which the less concentrated are diluted have been found to actually contribute to the working qualities of the ink to be produced.

CIRCULAR OF THE BUREAU OF STANDARDS, No. 185

UNITED STATES GOVERNMENT MASTER SPECIFICATION FOR STAMP-PAD INK

FEDERAL SPECIFICATIONS BOARD SPECIFICATION No. 166

This specification was officially promulgated by the Federal Specifications Board one June 30, 1924, for the use of the Departments and Independent Establishments of the Government in the purchase of stamp-pad ink.

I. Types.—Stamp-pad ink shall be of the following types: Black, blue, green, red, and violet.

II. Material and Workmanship.—Shall be as described under General Requirements.

III. General Requirements.—The ink shall not be inferior in any essential to one properly prepared accordingly to the following formula: Dissolve 5 grm of dye in 100 c.c. of 55% glycerin (specific gravity of the mixture 1.1415 at 20°/20° C.). Suitable dyes are the following:

	Schultz No.
Black—Nigrosine	700
Blue—Soluble blue	539
Green—Light green	505
Red—Magenta	512
Violet—Acid violet	530

IV. Detail Requirements.—Shall be as described under General Requirements.

V. Method of Inspection and Tests.—**1. Method of Taking Samples.**—Two fluid ounces of the ink, in an original unopened container bearing all of the manufacturer's marks, shall be sent to the testing laboratory.

2. Tests.—Equal volumes of the sample and of the standard ink of the same colour, prepared according to the above formula, shall be spread on equal areas of a new stamp pad, and impressions shall be made from them on white bond paper with a clean rubber stamp. The impressions shall be examined when fresh, and the time required for drying shall be noted. The sample shall take no longer to dry than the standard, and shall give as sharp and as intensly colored impressions.

The pads shall be exposed to atmospheric conditions for 10 days, and the tests shall then be repeated. During this time the sample shall show no more evidence of excessive hygroscopicity or of drying and caking on the pad than does the standard.

One-half of the sheet on which the impressions have been made shall be covered with black paper. The sheet shall then be exposed to direct sunlight for 48 hours, or at a distance of about 10 inches from an arc or ultra-violet light for 24 hours. The sample shall show no more evidence of fading than does the standard.

VI. Packing and Marking.—No requirements specified.

VII. Additional Information.—The ink is suitable for use with rubber stamps.

VIII. General Specifications.—No requirements specified.

MARKING INKS

Natural marking inks are used to a limited extent, and the Indian marking nut, *Semecarpus anacardium*, forms the basis of the pigment of some inks. Other natural marking inks and varnishes are obtained from the seeds of the avocado fruit, *Persea gratissima* and *P. drymifolia* (*J. Amer. Pharm.*, 1923, **95**, 612) and from the *poison ivy* and *poison sumach* (*Rhus toxicodendron* and *R. venenata*).

Silver Marking Inks.—The original inks required the use of two solutions—but subsequently a solution of silver tartrate in dilute ammonia was found more easy to use, and inks of this type are still on the market. The writing on the fabric is heated to reduce the silver salt to a black silver suboxide.

Aniline Marking Inks.—The action of the two-solution inks consists in the formation of aniline black within the fibres of the material. One solution contains a salt of aniline, and a thickening agent, and the other the oxidising agent (*e. g.*, sodium chlorate and copper chloride). The writing at first appears green, and gradually darkens on exposure to the air, the process being accelerated by heat.

There are also on the market one-solution aniline inks in which the final formation of the black pigment is suspended, and the oxidation does not take place until certain volatile constituents in the liquid have evaporated.

Examination of Marking Inks.—Naturally the behaviour of a marking ink is of greater importance than its composition. Tests to determine the following points should be made: 1. The ink must not injure the fabric; certain aniline inks are liable to make holes in the material when the marked place is heated.

2. The marking must be permanent, *i. e.*, it must not be bleached by boiling with soap or washing soda, and should resist the action of dilute sodium hypochlorite solution.

3. It must not change in the bottle; some aniline one-solution inks are liable to gelatinise suddenly when kept.

4. It must run smoothly from the pen, and yet not be so thin as to "run" when applied to the surface of the linen.

5. It should darken rapidly when heated (silver inks) or steamed and washed with soap and water (aniline inks).

BIBLIOGRAPHY: WRITING INKS

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PRINTING INKS

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Historical.—The first printing inks were made by incorporating lamp-black with burnt linseed oil. Later, to meet the requirements of new conditions of presses, paper, etc., other oils, such as rosin oil, mineral oil, the semi-drying oils, China wood oil, etc., were introduced, whilst gas black, bone black or ivory black, magnetic pigment, etc., replaced the original lampblack. Colour printing required a great variety of pigments, divided roughly into two classes, the inorganic pigments, and the coal-tar dyes and lakes. Still later is the development of an ink for printing from an intagilo plate, in the so-called "rotagravure" printing.

Types of Inks.—We recognise certain types of inks, which are useful for certain kinds of printing, and the requirements of the work as regards drying, viscosity, tack, and length[1] etc., dictate the ingredients to be used. These types, for convenience, may be listed as follows:

(1) *Web press ink;* for use in printing on a continuous sheet, such as newspaper work. High speeds are employed in such work, and the paper is invariably a wood pulp (largely mechanical), unsized paper. Such "drying" as takes place is not what we ordinarily mean by "drying" of an oil, but is the absorption of the ink by the paper. The ink is fed from an "overshot" fountain (or ink reservoir); that is, in such work, the ink is fed against a roller which moves upward from the ink, and carries the latter over to the distributing rolls which smooth it out to a thin, uniform layer for application to the type forms. Such a procedure requires a "long" ink; since a "short" ink would soon work away from the feed roll, and eventually stop the supply of ink being fed to the distributing rolls, and thence to the type.

Where drying by absorption takes place, such as in the web press type of ink, the oil portion of the ink will usually consist of mineral

[1] Cf. page 248, for definition of length in printing inks.

oil, kerosene, rosin and rosin oil. We do not expect to find any linseed oil or other drying oils, nor do we find the hard gums.

(2) *Flat bed inks;* these are the inks which are used on presses into which the sheets are fed singly. The paper is essentially of the same type as that used with web press inks, viz., a wood pulp paper, although it may be a better grade, and may have been calendered to a smoother finish. The speed is obviously slower than in the web press; hence we must look for some drying by oxidation to supplement the drying by absorption. We are, therefore, likely to find small percentages of linseed oil, and rarely some hard gums. The ink feeds downward from the ink fountain, so that these inks do not require the same degree of length as the web press inks—merely sufficient to ensure a continuous flow.

(3) *Job inks;* these are the inks used in printing on sized papers, such as the bonds, ledgers, etc. The penetration of the ink into the paper is, at the most, very slight, hence for drying we must depend entirely on the drying oils, and in order to secure the necessary tack to provide a bond between the paper and ink, we use the hard gum varnishes. Rosin and rosin oils are used in some of the cheaper grades, but even then form only a portion of the entire vehicle.

The non-drying oils cannot be present in any great amount, for such an ink would not dry rapidly, and hence would smear, or smut for a long time after printing.

(4) *Half-tone inks;* these are used in printing from cuts made from photographic plates. These cuts consist of a large number of irregular sized dots, some large and some small. The larger dots form the shading of the picture, and the smaller dots the lighter portion, or "high lights." The printing of illustrations for books is a good example of its use. The paper used is the so-called "coated" paper, the coating being either on one side, or both, as specified. This coating is usually a mixture of fine china clay and casein, and gives a peculiarly smooth glazed surface which readily takes the ink from even the finest and smallest dots in the half-tone cut. A very stiff ink is required, with good drying properties, but without much length. When these half-tones are inked, it is important that the ink be taken up only by the high spots of the cut; a long ink would pull into, and fill up the interstices between the dots, and the resulting printing would be greatly blurred. We therefore expect to find

a short ink, containing largely drying oils and drying varnishes, but without a great deal of tack.

(5) *Engraving inks;* these are the inks used in printing from an intaglio plate, such as is used in the printing of the common engraved visiting card, announcements of weddings, etc. The method for their use is to rub the ink over the plate so as to fill all the lines, remove the excess ink by rubbing with a rag, and then printing by pressing the plate against the card. This ink must be extremely short—quite butter-like in consistence, so that when the excess is rubbed off the plate, the rubbing will not remove the ink from the engraved lines. The drying is surface drying only, and the layer of ink is usually heavier than with any other type of printing, so that we must have the maximum of drying properties and even then it will be recalled that in most of this work, the ink is covered with slip sheets so that the ink, if it should offset slightly, will not damage the next card. The paper used is generally of the type known as Bristol board, and is a thick, heavy, strong paper, highly sized, and affording practically no opportunity for penetration.

(6) *Rotagravure inks;* these are quite a modern discovery, but their use is widespread, and has attained much popularity. Most of the Sunday newspapers in the United States have one or more sections which are printed with this type of ink. The printing is from an intaglio plate containing a large number of recesses, or cells. The depth of these cells determines the amount of ink to be transferred to the paper; the thicker the layer of ink, the darker it appears on the printed sheet, so that the light and dark portions are obtained by varying the depth of the cells, and so controlling the supply of ink. The ink is applied in excess to the plate, and the excess is removed with a scraper, or "doctor blade." Such inks must obviously flow very freely, be more or less translucent, and be absorbed rapidly by the paper. Only in this type of printing do we find the thickness of the layer of ink to vary from one part of the plate to another. There is no time for drying by oxidation, since this type of printing is comparable with the web press work. Tack is undesirable, as all of the ink in the cells of the plate must be transferred to the paper. These inks are unique, from the analyst's point of view, in that they are the only type of printing ink containing volatile oils in the vehicle. Opaque pigments are not used, since they do not readily lend themselves to shades of colour by varia-

tion in the thickness of film, and the colouring matter is usually a brown, bituminous substance, soluble in the vehicle.

(7) *Duo-tones;* these are really a special type of half tone or job inks, in which there is an oil-soluble colour present as well as the pigment. The ink is softer and more penetrating than the usual one-colour inks; the idea being that the oil will penetrate the paper, and spread out beyond the pigment, and thus give a second colour tone to the print. Brown and black, and green and black, are the favoured combinations.

Composition.—The components of a printing ink may be divided into two groups: (*a*) a vehicle, composed of various oils and gums; and (*b*), pigments, colours, etc.

The principal substances to be looked for in the vehicle are: linseed oil, China wood oil, soya bean oil, mineral oil, rosin oil, the semi-drying vegetable oils, certain bituminous substances, hard gums such as dammar and kauri, rosin (colophony), various potassium, sodium, calcium and aluminium soaps, and one or more of a variety of driers.

Most of these substances are used in printing inks without any special preparation, but others, notably the linseed oil, are given a special treatment to make them adaptable to the peculiar requirements of this type of work.

In the treatment of linseed oil, the older (and one still in use) is the burning process. The oil is heated to fuming, the fumes ignited, and the oil allowed to burn until it has attained the desired viscosity. The longer the oil burns, the stiffer will be the product. Such treatment produces a "short" oil, and one peculiarly adapted for half tone and engraving inks.

The terms "length" and "short" are in common use in the industry and should be defined. A long ink or varnish is one which will string out to a considerable extent without breaking off, whereas a short ink or varnish is one which will break off, and which possesses little adhesion. Honey or heavy molasses are common and familiar types of materials which we would describe as "long," whilst butter is of the type described as "short." This property of "length," or "short," is exceedingly important in the printing ink industry, since it refers to one of the most important working properties of the ink.

The second process for preparing linseed oil, is to heat the oil in an open kettle to about 300° without allowing the vapours to

inflame. By varying the time of heating, a number of grades are prepared, varying in viscosity and length. This process produces a long varnish.

These processes to which linseed oil is subjected, result in changing the constants of the oil, and the following figures, given by F. H. Leeds[1] show to what extent these changes have taken place:

Oil	Sp. G. 15°	Free acid %	Saponifiable %	Unsaponifiable %	Hübl iodine value %
Raw	.9321	0.85	288.	....	169.0
Tint	.9584	1.46	284.	....	113.2
Thin	.9561	1.76	285.	0.62	100.0
Middle	.9721	1.71	284.	0.85	91.6
Extra strong	.9741	2.16	294.	0.79	86.7
Burnt thin	.9675	6 93	287.	1.35	92.7

The principal changes to be noted are: the gradual increase in the specific gravity and free acid; practically no change in saponifiable or unsaponifiable: the rapid drop in the iodine value; and the unusual rise in free acid and unsaponifiable in the burnt oils.

The commonly accepted methods for estimating these constants are applicable to these so-called "linseed varnishes," except the methods for the iodine absorption. The work of Smith and Tuttle[2] demonstrated that the methods, as ordinarily defined, allow too much leeway to permit of obtaining accurate results with these special types of linseed oil, and the following procedure is therefore recommended:

Prepare the Hanus solution by dissolving 13.2 grm. of iodine in 1000 c.c. of glacial acetic acid (99.9%), and then add 3 c.c. of bromine. Allow the solution to stand for several days before using.

The sodium thiosulphate solution for use in titrating the excess of iodine, should be tenth normal, and freshly standardised against potassium dichromate.

Weigh accurately 1 grm. of oil into a 100 c.c. graduated flask and add chloroform to the mark. Take 10 c.c. of this solution, representing 0.100 grm. of oil, and transfer to a stoppered flask or bottle; add 25 c.c. of the Hanus solution, insert the stopper, and transfer the flask to a cool, dark closet. At the end of exactly 30 minutes, add 25 c.c. of 10% potassium iodide solution, 100 c.c. of distilled

[1] *J. Soc. Chem Ind.* 1894, 13, 204.
[2] Technologic paper, 37, U. S. Bureau of Standards; *J. Ind. Eng. Chem.* 1914, 6, 904.

water, and a few drops of a freshly prepared starch solution. Titrate at once, as rapidly as possible. Calculate the iodine absorbed by the oil in the usual way.

By referring to the results obtained by Smith and Tuttle, it will be noted that it is of vital importance, in order to secure comparable results, that there should be as little variation in the amounts of material used, and in the time, as is possible. The temperature must be held reasonably constant, and 25° is recommended.

It is very important to remember that no single part of the procedure may be changed, without causing a considerable variation in the results, and unless one is willing to adhere strictly to the prescribed routine, comparison of results with previously obtained data is simply impossible.

Varnishes.—The term varnish is sometimes applied to the special linseed oils, but properly, it should be applied to the combinations of rosin or the hard gums with some oil. Rosin varnishes are prepared by heating rosin and rosin oil together, whilst the hard gum varnishes are prepared by heating linseed oil with dammar or kauri. The viscosity of the varnish will depend upon the quantity of gum and the type.

The principal reason for preparing these varnishes separately is that it facilitates the mixing and incorporating of these hard gums in the ink.

Driers.—The driers form an extremely important feature of the vehicle. The principal ones are the borates, resinates, and linoleates of lead and manganese. They may be added directly in the ink formula, or as a so-called "Japan drier," which is a mixture of the desired lead or manganese compound with varnish gum, linseed oil, and turpentine. The percentage of drier used is so small that the turpentine becomes a negligible feature of the ink, and is not estimated by the analyst.

Pigments.—The commonly used pigments, not necessarily arranged in the order of their relative importance, are:

Carbon black, or Gas Black, is the finest black obtainable. It is made from burning natural gas with an insufficient supply of oxygen, and collecting the soot. It is practically pure carbon, without ash or oil. It has greater covering power than any other pigment used in printing inks.[1]

[1] For suggested specifications for carbon blacks for printing inks see Perrott, Bull. No. 192, Bureau of Mines, Dept. of Interior, U. S. A., 1922, p. 65.

Lampblack is also practically pure carbon, but is not as finely divided as carbon black. It is practically free from ash, but usually contains a certain percentage of oil. It is prepared by burning, with an insufficient supply of oxygen, some organic material such as rosin, or rosin oil, mineral oil, etc. The soot is caught in a series of chambers, thus yielding several grades, the heavier and coarser being naturally in the chamber nearest the fire. Lampblack is sometimes given a heat treatment to reduce the amount of oil or grease it may contain.

Vine black was originally the charred remains of vines or twigs, but it has now been extended to include the charred remains of many organic substances, such as fruit pits, and nut shells. The product is ground to the desired fineness. This black is likely to contain a fairly high ash content.

Bone black is the charred remains of ivory or bone. It is much coarser than the previously mentioned blacks, and yet, peculiarly enough, it is the deepest, densest black, and is quite important in the preparation of engraving inks, where its depth and tone give desirable properties to the ink, and where its lack of covering power is unimportant.

Magnetic black is a black prepared by a patented process, and is essentially finely divided magnetic oxide of iron.

Whiting should be practically pure carbonate of lime, soft, and free from grit.

Barytes comes in two types, the precipitated, commonly called "blanc fixe," and the ground. The former grades have the greater covering power, are softer and more likely to be free from grit. The ground barytes have little to recommend them, other than price, for the manufacture of printing inks.

Lithopone is a mixture of zinc sulphide and barium sulphate, obtained by the interaction of zinc and barium salts. The better grades are those carrying the higher percentages of zinc sulphide. Lithopones have a good white colour, and excellent covering power. On account of the sulphide, they are not used where lead driers are to be used, nor can they be used with chrome green.

White lead is the common, well known basic carbonate of lead. Its properties are too well known to require further description.

Zinc oxide is the fumed pigment obtained by roasting zinc ores or spelter. Its covering power is second only to that of carbon

black. It should be pure zinc oxide, free from grit, and from other metals.

Prussian Blue is the generic term for the various blues produced by the interaction of an iron salt and an iron cyanide. Its importance in ink manufacture is owing to the fact that in the preparation of black inks, a certain percentage of blue is necessary to neutralise the yellow of the vehicle, and yield a clear, dense black. Either Prussian blue, or blue dyes, or both, may be used for the purpose. The term Prussian blue is used here to denote any of the iron cyanide blues such as Milori, Bronze, Chinese Blue, etc., which may be used in printing inks. These names are applied more or less indiscriminately; the simplest procedure is to use the better known term "Prussian blue" to cover all of them. Even if there were a standard nomenclature, it is doubtful if the various blues could be identified after incorporation in a printing ink.

Ultramarine blue is an artificial blue prepared by heating together China clay, sodium carbonate or sulphate, carbon and sulphur. Its use is confined largely to the preparation of coloured inks.

Chrome Green, i. e., the true chrome green, is of course, the trioxide of chromium, but this pigment is rarely used in printing inks. What is usually meant by chrome green in the printing ink industry is the green produced by the mixing of Prussian blue and lead chromate. There will be little difficulty in discerning when the true chrome green is used; the absence of sufficient lead to unite with the chromium and the presence of iron in the ash will suffice.

Vermilion, or as it is more commonly known, English Vermilion, is the red sulphide of mercury. The pigment should be examined for adulterants such as organic lakes and dyes. Extraction with alcohol, ethyl ether, and benzene, will detect the dyes, whilst the lakes will be detected through the ash of the pigment. True English vermilion should have practically no ash.

Chrome yellow is a yellow chromate of lead, and is a much used and quite important pigment in the ink industry. It comes in a number of hues, varying from lemon-yellow to orange or even scarlet. The former will contain an excess of lead sulphate, and the deeper colours varying amounts of basic chromate.

Earthy pigments will include the clays, siennas, umbers and ochres, and possibly also such iron pigments as Venetian Red. Their

examination should be conducted so as to show covering power and freedom from grit.

Organic colours and *lakes* are used extensively. The blue dyes are much used with Prussian blue for toning black inks. The others find their greatest use in colour work.

Manufacture of Ink.—The mechanical part of the manufacture of printing inks is quite simple. The vehicle is prepared according to the desired formula and then the pigments are added. There is usually a preliminary mixing in a mill containing broad revolving knives or paddles, after which the inks are ground in a grinding mill. This consists of three rolls which revolve at different speeds, the rear one slowest, the front roll fastest. The ink is fed between the rear and middle rolls, and is carried around by the middle to the front roll, where it is automatically scraped off. The differential speed gives the grinding effect and reduces the pigment to the finest division possible. Many of the better grades of ink are ground a number of times before they are considered satisfactory.

Analysis.—There has been very little work published on the analysis of printing inks. The methods which are given below are taken largely from the article on this subject by J. B. Tuttle and W. H. Smith.[1] The general procedure consists in separating, by means of suitable solvents, the inks into two parts, oils and pigments, and testing the separate parts for the constituents which are likely to be present.

For inks in which the vehicle is largely, if not entirely, mineral or rosin oils, petroleum spirit will be found a very satisfactory solvent, but for general use where the composition is unknown, a mixture of 3 parts ethyl ether and 1 part benzene is to be preferred. The separation is best performed by centrifuging; the settling process consumes too much time to be practicable.

Separation of Oil from Pigment.—About 50 grm. of ink (avoiding the hard film which frequently forms on the surface) are placed in a weighed glass tumbler of about 300 c.c. capacity, a small amount of solvent added, and the whole stirred thoroughly until a homogeneous mixture is obtained. The glass is then filled with the solvent to within about ½ in. from the top, and the whole again stirred. It is next placed in the metal cup of the centrifuging machine and the space between the glass and metal cups filled with water in order to

[1] *Technologic Paper No. 39, of the U. S. Bureau of Standards,* "The Analysis of Printing Inks."

equalise the pressure of the liquid inside the glass during the centrifuging. Placing a rubber disc at the bottom of the metal cup has been found to materially lessen the danger of breaking during the operation. The metal cup and contents are then exactly counterbalanced, most conveniently by either a second sample of the same ink or another sample of ink, and then both are placed in the machine. For web-press and flat-bed inks, 2000 revolutions per minute for 10 minutes will suffice for a complete separation. Where gas black has been used, it has frequently been necessary to run the machine at 2600 to 2800 revolutions per minute for 20 or 30 minutes before a satisfactory separation is secured. The clear liquid is decanted through a pleated filter into a glass bottle, a further quantity of solvent added, and the process repeated. Usually three treatments suffice to give practically complete separation of oil and pigment. The glass and contents are dried at about 90° and on cooling, reweighed. The increase in weight is the pigment, which is calculated as a percentage. The amount of pigment on the filter paper should be negligible if the centrifuging has been efficient.

This method will not always yield results of great accuracy. The errors, which vary in magnitude with different inks, are as follows:

Some of the dyes are soluble to some extent in the solvents, tending to give low results for pigment.

Hard gums may not be completely soluble, and thus part will remain with the pigment.

The hard scum (linoxyn), which forms on the surface of the ink after it has been exposed awhile, is difficultly soluble and remains with the pigment. This should be excluded in sampling, for if it is not done, a considerable error may be introduced.

Carbon black contains some particles so fine that it is impossible to cause them to settle, even in the centrifuge.

The net error of this separation is therefore the algebraic sum of these various errors.

The usual types of printing inks have little or no volatile portion, so that there is no loss by the above method. Rotagravure inks, however, have a volatile portion, and in order to estimate it, proceed as follows:

Centrifuge a weighed portion of the ink as above, using benzene-ether solvent if necessary. Decant from any fillers which may be present, dry the pigment and the vehicle to constant weight (approxi-

mately). The difference between the sum of the non-volatile vehicle and the pigment, and the original weight of the ink used, may be called "volatile vehicle."

If it is desired to examine this volatile vehicle transfer a weighed portion of the ink to a small glass distilling flask, and distil over the volatile portion. The amount thus collected may be checked against the value obtained by centrifuging.

Analysis of the Oil.—The oil fraction may contain any of the oils, etc., mentioned in the paragraph on the composition of inks. Bituminous substances are judged largely by colour, being a mixture of a number of different substances of varying chemical nature; the estimation of the total amount present is a matter of too much difficulty to justify the time required.

Oil Constants.—Estimating the oil constants, such as iodine value, saponification value, acid value, etc., does not give very reliable data regarding composition. If there were but two components, the proportion of each might be estimated at least approximately, in this way, but with three, and sometimes more substances present, such methods are useless, even if the constants of the individual substances are well known.

We are therefore forced to rely upon qualitative tests, supplemented by quantitative estimations of some of the more important constituents. The oil fraction of an ink is independent of the colour; therefore the separation given below is applicable to inks of all colours.

It will be found convenient to regard the oil fraction as consisting of hard gums, rosin, unsaponifiable matter (rosin and mineral oils) and linseed oil.

Hard Gums.—The hard gums are difficult to estimate, the only method which has given any measure of satisfaction being that of McIlhiny.[1] This method depends upon the insolubility of hard gums in water and petroleum spirit. The method is much better adapted for the analysis of paints than printing-ink varnishes, but it can be used for the latter to obtain some idea of the amount present.

Unsaponifiable Matter.—Sufficient of the solution from the separation of the oil and pigment to leave a residue of about 5 grm. is evaporated in a weighed beaker; 50 c.c. of normal alcoholic potassium

[1] P. C. McIlhiny, *Chem. Eng.*, 1908, **8**, 70; *Chem. Abs.*, 1908, **2**, 2630.

hydroxide are added, the beaker covered with a watch glass, and heated on a steam-bath for several hours, being stirred frequently to assist saponification. When this is complete, the watch glass is removed and the alcohol distilled off. The residue is transferred to a separating funnel with successive portions of water, in all about 100 c.c. being used, and extracted with petroleum spirit until no further oil can be removed. Four extractions are usually sufficient. The petroleum spirit fractions are united in another funnel, washed with water until the wash-water is no longer alkaline, filtered into a weighed beaker, the petroleum spirit distilled off, and the residue dried at 95°, cooled and weighed. If this unsaponifiable matter is over 2%, it indicates the presence of something else than linseed oil and hard gums. The wash-water from the first two washings should be united with the water layer in the first separating funnel.

Rosin.—This unsaponifiable matter is tested for the presence of rosin oil. The most satisfactory method of testing qualitatively for this material is the Liebermann-Storch test, which consists in heating a small portion of the oil with 10 c.c. of acetic anhydride, allowing it to cool to room temperature, and adding a drop of sulphuric acid of sp. gr. 1.63. A violet coloration indicates rosin oil. It is always best to carry out a control test at the same time with some pure rosin or rosin oil. The test is identical for the two materials. This test is best carried out by using the unsaponifiable portion of the vehicle.

If the test for rosin oil is positive, the alkaline aqueous solution which has been extracted with petroleum spirit is made acid with hydrochloric acid (there is usually sufficient dye present from the ink to act as indicator), and the fatty acids which are thus liberated are extracted with successive portions of ethyl ether. These extracts are united, washed free from acid and salts and evaporated in a small beaker.

A quantitative estimation of the rosin can be made either by the Twitchell method, which depends upon the separation of the esters of the organic acids, or by Gladding's method,[1] which depends upon the separation of the silver salts of these acids.

A very satisfactory method is Parry's modification of Gladding's method. The fatty acids are dissolved in 20 c.c. of 95% alcohol,

[1] *Amer. Chem. Jour.*, 1881, 3, 416.

a drop of phenolphthalein added and then a strong solution of sodium hydroxide (1 part alkali to 2 water) until the reaction is just alkaline. The solution is heated for a few minutes, allowed to cool, and then transferred to a 100 c.c. stoppered graduated cylinder. The latter is filled to the 100 c.c. mark with ethyl ether, 2 grm. of powdered silver nitrate crystals are added, and the mixture shaken vigorously for 15 minutes, in order to convert the acids into their silver salts. When the insoluble salts have settled, 50 c.c. of the clear solution (containing the silver salts of rosin) are pipetted off into a second 100 c.c. cylinder, and shaken with 20 c.c. of dilute hydrochloric acid (1 acid to 2 water). The ethereal layer is drawn off, and the aqueous layer is shaken twice with ether. The ether extracts are united, washed with water, and the ether distilled off in a weighed beaker. The residue (rosin) is dried at 110° to 115°, cooled, and weighed. The results are calculated on the basis of the original weight of the oil.

The difference between 100 and the sum of the unsaponifiable matter (if over 2%) and the rosin, may be considered linseed oil.

Analysis of the Pigment

Black Inks.—A mixture of oil and black pigments will not give a pure dense black, owing to the various undertones of the pigments. Moreover, the public is accustomed, in printing, to accept as black what is really a blue-black. Practically all of the pigments from black inks will be found to contain more or less blue, either in the form of Prussian blue, or blue dyes and lakes.[1]

Ashing.—The first step in the analysis of the pigment of a black ink is to ignite a weighed quantity in a porcelain crucible (platinum cannot be used on account of the lead which is usually present). The ignition should be performed at the lowest possible temperature required to obtain complete combustion. This precaution is general and applies to all inks. The loss on ignition represents lampblack, the carbon of the bone-black (should there be any present), aniline dyes, and undissolved oils and gums. Prussian blue is decomposed by heat, part of it being volatilised, the iron remaining behind as ferric oxide. The residue from the ignition contains any added mineral matter of the pigment, lead or manganese from

[1] For recent analysis of lamp blacks and gas blacks see Selwig, quoted by Neal and Perrott, Bull. 192, Bureau of Mines, Dept. of Interior, U. S. A., 1922, p. 72.

the driers, ferric oxide from the Prussian blue, or ferric oxide added as such (the so-called magnetic pigment), calcium phosphate if bone-black is present, and alkali or calcium carbonates from the soaps present. All ignitions of pigment must be performed under a hood having a strong draught.

Prussian blue should be tested for qualitatively in the dry pigment. For this purpose, 1 grm. of pigment is moistened with 2 or 3 c.c. of normal alcoholic potassium hydroxide, heated on the steam-bath until the alcohol is removed, 5 c.c. of water added, and the insoluble matter filtered off. The filtrate is made acid with hydrochloric acid and filtered again if necessary. When ferric chloride is added a blue precipitate will be obtained if Prussian blue is present. Sometimes sufficient blue dye goes through the filtrate to obscure the indication. In this case the solution is again made alkaline and filtered. After filtration it is made acid with hydrochloric acid as before, and then copper sulphate is added. The precipitate is filtered off and washed thoroughly; it consists of reddish-brown copper ferrocyanide. It is advisable, in case of doubt, to add a small amount of Prussian blue to the pigment, and make a control test. The ash is analysed quantitatively for insoluble matter, lead, iron, manganese and calcium.

Insoluble Matter.—0.250 grm. of the ash is heated to dull redness in a porcelain crucible for a few minutes, cooled in a desiccator, and weighed. It is transferred to a 250 c.c. beaker, concentrated hydrochloric acid being used to dissolve any material that may stick to the crucible. About 25 c.c. of concentrated hydrochloric acid are added, the beaker covered with a watch glass, and after being heated until as much as will go in solution is dissolved, the cover is removed, and the solution evaporated to dryness. The residue is moistened with a few drops of concentrated hydrochloric acid, 50 to 75 c.c. of boiling water added, the solution is filtered, and the residue thoroughly washed with hot water. The filter paper and residue are ignited and weighed, and the product called "*insoluble matter.*"

Lead.—50 c.c of 10% sulphuric acid are added to the filtrate from the previous estimation and evaporated down until the solution fumes strongly. This is cooled, diluted carefully with about 100–150 c.c. of water and heated on the steam-bath until any basic ferric sulphate which sometimes separates is redissolved. The precipitate,

containing the lead sulphate, is now filtered off. A small amount of lead sulphate will, in all probability, remain in solution, but inasmuch as the ash is seldom more than a few per cent. of the entire ink, and of this only a small amount is lead, the amount thus lost is negligible. The precipitated lead sulphate is dissolved in ammonium citrate or acetate solution, filtered from any insoluble matter, the filtrate made strongly acid with sulphuric acid, and the precipitated lead sulphate filtered off on a Gooch crucible, ignited and weighed. A platinum Gooch crucible with a platinum felt will be found extremely satisfactory. The insoluble matter from the ammonium acetate solution should be examined for calcium and barium.

Another method for the estimation of the lead is nearly to neutralise the acid present with sodium carbonate, saturate the solution with hydrogen sulphide, filter off the precipitated lead sulphide, dissolve it in fairly strong nitric acid and determine the lead as sulphate by adding sulphuric acid as above described. In this case solution in ammonium acetate is omitted. The former method is advantageous when qualitative tests show that there is very little manganese present, and it is desired to estimate only the iron. After the lead sulphate has been removed the solution obtained is in perfect condition for this estimation.

Iron.—The iron in the filtrate from the lead sulphate is reduced to the ferrous condition by passing the solution through a Jones reductor, and the ferrous sulphate titrated with a standard solution of potassium permanganate.

Iron is separated from manganese and other metals which may be present by precipitating with ammonium hydroxide, the precipitate being filtered off, redissolved in hydrochloric acid, reprecipitated with ammonium hydroxide and again filtered. It is now dissolved in hydrochloric acid, sulphuric acid added, and the solution evaporated until all the hydrochloric acid is removed; it is diluted and the iron estimated as before, with the Jones reductor. This method is rapid and accurate. Before adding ammonium hydoxide, if hydrogen sulphide has been used, the solution should be boiled until all the hydrogen sulphide is removed, and nitric acid added to oxidise the iron to the ferric condition.

Manganese.—Hydrogen sulphide is now passed into the ammoniacal solution from the iron precipitation. This is allowed to stand over night, and the precipitate, if there is any, is examined for

manganese. Usually there is only a trace of manganese, insufficient to warrant a quantitative estimation. Should there be much manganese, the sulphide can be filtered off, and the quantitative estimation made by conversion into the pyrophosphate.

Calcium.—If it is desired to estimate the calcium, this can be done after filtering from the manganese sulphide. (If phosphates are present, as for instance, if bone-black is present, a basic acetate separation is required.) In either case, the lead should be separated by hydrogen sulphide. The filtrate from the manganese sulphide is heated on the steam-bath until the hydrogen sulphide is removed, ammonium hydroxide and ammonium oxalate are added, and the precipitated calcium oxalate is estimated either as calcium oxide or sulphate.

Nature of the Pigment.—The percentage of ash will be of great assistance in determining the nature of the pigment. Black oxide of iron is only slightly changed on heating, being completely oxidised to ferric oxide. Bone-black is composed largely of calcium phosphate, yielding the greater part of its weight as ash. The presence of any large amount of phosphoric acid will be sufficient evidence that bone-black has been used.

In the absence of black oxide of iron, we may assume that all of the iron in the filtrate is due to the Prussian blue. The percentage of Fe_2O_3 in the ash, multiplied by the percentage of ash in the pigment, multiplied by the factor 1.53, will give roughly the amount of Prussian blue present. The factor 1.53, is obtained from the ratio $Fe_7(CN)_{18}$ to Fe_2O_3. It is purely theoretical and is probably low, but is sufficiently accurate for most purposes.

When the presence of oxide of iron is suspected, 1 grm. of pigment is wrapped in filter paper, and the dye extracted with alcohol, in an extractor of the Wiley type, in which the material is extracted by the solvent at its boiling point. When all the dye has been extracted, the paper and contents are dried, and the nitrogen is estimated in the residue by the Kjeldahl method. From the nitrogen thus obtained, the Prussian blue is calculated, the factor 3.41 being used. The Fe_2O_3 present in this amount of Prussian blue is deducted from the total Fe_2O_3 found in the ash. The remainder will be the percentage of iron from the magnetic oxide. The formula of the latter is theoretically Fe_3O_4, and the proper calculation should be made. This method for the estimation of Prussian blue depends

upon the fact that the aniline dye is the only other material which may contain nitrogen. Instead of calculating the Prussian blue from the amount of iron present, it is estimated from the nitrogen remaining after the removal of the aniline dye. In this way both Prussian blue and magnetic oxide of iron may be estimated with reasonable accuracy.

Dyes.—Practically all the dyes which are used in black printing inks are soluble in alcohol, so that an approximate estimation can be made by extracting the pigment with this solvent. This method is the same as described in the preceding paragraph, the alcoholic solution being evaporated off in a weighed beaker, the residue dried at 90°, cooled and weighed.

Blue Inks

A weighed quantity of pigment is ignited as described under black pigments. The ash is analysed by the same process as before, only lead, manganese, and iron being estimated if the qualitative tests show that Prussian blue is present. The lead and manganese are reported as metallic driers, the iron is calculated as Prussian blue, and the remainder reported as mineral filler. The composition of the filler, as a rule, is of no consequence.

Ultramarine.—The presence of ultramarine will be shown by the blue colour of the ash. Hydrogen sulphide is evolved from the latter on the addition of hydrochloric acid. There is, unfortunately, no method for its quantitative estimation. In this case, the ash is reported after deducting the lead and manganese.

Soluble aniline dyes are estimated by extraction with alcohol as under black pigments.

Red Inks

Vermilion.—The most brilliant red mineral pigment is unquestionably vermilion (mercuric sulphide). Its price prohibits its use except in inks used for special purposes. It is very readily detected qualitatively by covering a small quantity of pigment with 4 or 5 c.c. of *aqua regia,* and heating gently. The solution is diluted with 5 volumes of water, filtered, and stannous chloride added to the filtrate. A grayish precipitate of mercury will be formed if vermilion is present. A very small amount can be readily detected by this test.

The quantitative estimation of vermilion, however, is much more difficult. One method is to dissolve the mercuric sulphide in *aqua regia*, and after nearly neutralising the diluted solution, to precipitate the mercuric sulphide with hydrogen sulphide and weigh the precipitate on a Gooch crucible, observing all the precautions to eliminate sulphur which separates during the precipitation.

The following process has also been found of value: 1 grm. of the pigment is treated with a slight excess of ammonium sulphide. Sodium hydroxide is then added, while stirring. The beaker is placed upon the steam-bath, more alkali is added if necessary, until all the mercuric sulphide passes into solution. An undue excess of alkali should be avoided. The solution is allowed to cool, filtered, and the residue washed thoroughly. Sufficient ammonium nitrate to reprecipitate the mercuric sulphide is added to the filtrate, and it is then boiled to expel ammonia. The precipitate is allowed to settle, which takes but a short time, and the supernatant liquid decanted through a weighed Gooch crucible. The residual mercuric sulphide is boiled with a little sodium sulphite solution to remove free sulphur, and is then transferred to the crucible, where it is washed with hot water until it no longer reacts with silver nitrate solution. It is dried at 110° and weighed.

The distillation method, in which the mercury is absorbed by gold, and the various electrolytic methods will appeal to those who have had experience with them.

Metallic Driers.—The pigment is ignited and the ash analysed for lead and manganese. The remainder of the ash is reported as mineral filler.

Green Inks

The colouring matter may be chrome green, green lake or dye. Some of the darker shades are obtained by the addition of lampblack.

Ash.—The ash of the pigment is estimated as usual. Part of this ash is tested qualitatively for chromium. If present, the ash should be tested for the following substances: lead chromate, lead sulphate, lead oxide, barium sulphate, calcium sulphate, ferric oxide, and oxides of manganese.

Sulphur.—To estimate sulphur, 0.25 grm. of the ash and 5 grm. of a mixture of equal parts of potassium nitrate and sodium carbon-

ate are fused in a porcelain crucible over a sulphur-free flame. The cooled mass is extracted with hot water and filtered. The filtrate is acidified with hydrochloric acid, heated to boiling, and 10 c.c. of 10% barium chloride solution are added. After standing over night, the precipitated barium sulphate is filtered off, ignited, and weighed as usual. The solution should be sufficiently acid to prevent any significant contamination of the barium sulphate with barium chromate.

Barium.—To estimate barium, the insoluble matter from the estimation of sulphur, is dissolved in hydrochloric acid, the solution made nearly neutral with sodium carbonate, and hydrogen sulphide is passed into the solution until all the lead is precipitated. The lead sulphide is filtered off, the filtrate heated to boiling, and 10 c.c. of 10% sulphuric acid are added. The barium sulphate is treated as directed under the estimation of sulphur.

Chromium.—A fresh portion of ash is mixed with sodium peroxide, and fused in a nickel crucible. The cooled melt is dissolved in hot water and filtered. Carbon dioxide is passed into the filtrate, and the latter heated again on the steam-bath in order to precipitate any lead which may have been held up by the sodium hydroxide. Any insoluble matter which may separate is filtered off. The filtrate is made strongly acid with hydrochloric acid, potassium iodide added, and the liberated iodine titrated with a standard sodium thiosulphate solution. From the amount of thiosulphate used, the amount of chromium oxide present is calculated.

The two precipitates from the estimation of chromium are combined, and used for the estimation of lead, iron, manganese and calcium. They are dissolved off the filter paper with hydrochloric acid, the solution is nearly neutralised with sodium carbonate, and hydrogen sulphide passed into the solution. The precipitated lead sulphide is filtered off, dissolved in nitric acid, and estimated as sulphate as directed under black pigments. The filtrate from the lead sulphide is treated for iron, manganese, and calcium, as directed under black pigments. Usually only the iron is of sufficient importance to warrant a quantitative estimation.

It is difficult to give precise directions for calculating the results from the preceding estimations. To a large extent the analyst must use his experience in deciding the various questions as they arise. It is probably safe to assume that all of the chromium was originally

present as lead chromate,[1] and it should be so calculated. The iron oxide should be calculated as Prussian blue, provided there is a positive qualitative test. Any barium present should be calculated as sulphate; if there is any question as to its being originally present as carbonate, the ash of the pigment is treated with very dilute hydrochloric acid, the solution filtered, and the filtrate tested for barium. Barytes is difficultly soluble in cold dilute hydrochloric acid. In the absence of barytes, the sulphur present is calculated as lead sulphate. The excess of lead over that required for the lead chromate and sulphate, may be considered as drier.

China clay may be present, either as an added part of the chrome green, or as the base of a green lake. Aluminium hydroxide is also used as a base for coal-tar lakes. In such cases, the unestimated portion of the ash should be reported as lake base or mineral filler.

Green dyes are estimated by extraction as usual. In the absence of chrome green, the pigment is ashed, and the ash analysed for lead and manganese only, the remainder being reported as mineral fillers.

If lampblack has been used to produce a dark shade of green, it can be tested for qualitatively by taking a small portion of the pigment, treating it with strong alkali, and filtering through a Gooch crucible, washing first with hot water, and finally with moderately strong hydrochloric acid. Lampblack will show a black residue, which will disappear on ignition. No quantitative estimation has been developed for this material, and it is generally classed with the volatile constituents, which are then reported as aniline dye, lamp-black, undissolved oil, etc.

Inks of Other Colours

The above classes represent the inks most used for ordinary work. If it is desired to test inks of other colours, the general procedure would be simply to make qualitative tests for the pigments. The metallic driers present can be estimated in the ash if so desired. Reference to the various text-books on this subject may be of assistance in suggesting what materials may be present.

Permanence to Light

With coloured inks the question of importance is frequently not so much what dye or lake has been used, and how much, but how

[1] If there is only a very small percentage of lead present, it is probable that the chromium was present as the green oxide of chromium CrO_3, and should be so calculated, but such cases are rarely met with in printing inks, largely owing to the higher cost of the chromium oxide pigment. The principal advantage of the latter over the lead chromate and Prussian blue pigment is that the chromium oxide may be used in the presence of sulphur.

permanent it is. Exposure to light is the easiest method for determining this. This test is performed by making some streaks on white paper with the ink in question. These should be about ½ in. wide, and about 10 in. long. The film of ink should be as thin as it is possible to make it, and should correspond as nearly as possible to the thickness of the film of ink used in printing. The sheet is allowed to remain in a dark place for 24 hours to dry thoroughly, and is then divided into three parts. The middle section is exposed to direct sunlight until the colour changes, or until it is apparent that no change will take place, 50 to 75 hours being about the right length of time. The two outside sections are kept in the dark, for the purpose of comparison. After the exposure is completed, the strips are joined together in their original position, when it is possible to detect very slight changes in colour. A number of inks can be tested on the same sheet if so desired.

Another method for determining the relative permanence of different samples of the same colour has been suggested.[1]

Flat tints of each ink are printed as strongly as if they were to be part of a colour job. These tints should be about 5 in. by 7 in. in size. They should then be cut out to this size. A photometer scale is then made of five layers of fine tissue paper, each layer 1 in. narrower than the preceding. This will give five different thicknesses of tissue, each thickness representing a band 1 in. by 7 in. Across these, and about 1 in. apart, should be glued three strips of opaque black paper, 1 in. wide and 5 in. long, starting 1 in. from and parallel to one narrow edge on the tissue paper. The photometer thus made, and a printed sheet of the ink to be tested, are then put into a photographic printing frame which has a plain glass in the front of it. The whole is then exposed to the sunlight with an ordinary photographic printing-out photometer until the total exposure has reached a certain value on the photometer scale (usually the last number). When the printed sheet is taken from the frame, it will be found to be divided into three unfaded areas, corresponding with the three opaque black strips, and four faded areas, each divided into five 1-in. squares which have each received different amounts of light. By placing sheets of different printing inks of the same colour in the frame, and exposing to sunlight to the same photometer number, the

[1] Private communication from Mr. H. R. Gaylord and Mr. Averill, of the State Institute for the study of Malignant Disease, at Buffalo, N. Y., through Mr. E. S. Moores of the U. S. Government Printing Office at Washington, D. C.

relative permanence of the different inks can be seen at a glance. If desired, three inks may be tested at one time by cutting the flat tints into strips 2 in. wide and 5 in. long, and placing them in the frame so that each has an exposed and an unexposed area.

Dyes and Lakes

So far, little success has been met with in attempting to estimate the various coal-tar lakes used in printing inks. The soluble dyes may be removed by extraction, and the amount present estimated in this way. Some of these may be identified by the number and location of the absorption bands, using for this purpose the tables given by J. Formanèk in his book on "*Spektranalytischer Nachweis Künstlicher Organischen Farbstoffe.*" Other references are given in the bibliography. See also Fièrz, Vol. VI and The Colour Index.

Special Tests

The foregoing tests cover practically all the important components of the common inks. A few other tests might be made in case of trouble that cannot otherwise be located.

Volatile constituents in the ink can be estimated by placing a weighed quantity in a shallow layer in a porcelain or glass dish and heating in an air-bath for 1 hour at 105°, cooling in a desiccator, and weighing. It is hardly necessary to take the precaution of drying in an inert atmosphere. These volatile constituents may be benzine, turpentine, benzene, etc.

Certain patents call for the use of sodium silicate (water glass) in the thickening of the oil. The alkaline nature of this substance would prohibit its use in the presence of blue dyes and Prussian blue. It will probably be found with the pigment, and is easily tested for by treating the pigment with boiling water, filtering off the undissolved material, and testing the filtrate with phenolphthalein. It can hardly be considered a desirable substance in printing inks.

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AMINES AND AMMONIUM BASES

REVISED BY H. E. COX, M.Sc., Ph.D., F.I.C.

The amines are substituted ammonias of the type

NH_2R	NHR_1R_2	$NR_1R_2R_3$
Primary.	Secondary.	Tertiary.

derived from ammonia by the replacement of its hydrogen atoms by alkyl or aryl groups, *R*. When more than 1 hydrogen atom is replaced, the alkyl groups introduced may be identical, as methyl for example, in trimethylamine, NMe_3, or different, as in methyl-ethyl-propylamine, NMeEtPr. In the latter case the amines are known as *mixed* amines.

From the commercial point of view the most important amines are those of the aromatic series, such as aniline, naphthylamine, etc. It is usual, however, to distinguish these from the aliphatic amines, from which they differ in certain important respects, by giving them the name of *aromatic amino-compounds*, the true aromatic amines being substances in which the nitrogen is attached to the aliphatic residue, as, for example, benzylamine, $NH_2.CH_2.C_6H_5$. The aromatic amines bear a much closer relationship to the aliphatic amines than the coal-tar bases, of which aniline is the most important representative, in which the nitrogen is attached to the benzene ring.

The aliphatic amines and many of the coal-tar bases have an alkaline reaction and may be directly titrated with acids in the presence of a suitable indicator; also, they form complex salts, platinichlorides and aurichlorides, with platinic or gold chloride such as methylamine platinichloride $(CH_3NH_3)_2PtCl_6$, aniline aurichloride $C_6H_5NH_2HAuCl_4$. The salts have definite m. p. and crystalline form; they are usually not very soluble and hence are available for the recognition and estimation of many of the amines.

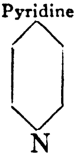
Diamines are derived from hydrocarbons by replacing 2 hydrogen atoms by 2 amino-groups, *e. g.*, *ethylene-diamine*, $NH_2.CH_2.CH_2$.-

NH_2; *phenylene-diamine*, $NH_2.C_6H_4.NH_2$; *tetramethylene-diamine* (*putrescine*), $NH_2.CH_2.CH_2.CH_2.CH_2.NH_2$.

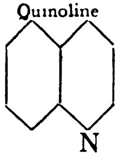
Triamines or *triamino-compounds*, *tetramines* or *tetramino-compounds* are also known but are relatively unimportant.

Ring nitrogen compounds such as pyridine and quinoline have the

Pyridine

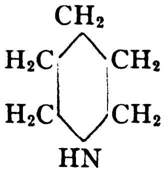


Quinoline



properties of tertiary bases. The reduced pyridine, hexahydropyridine

or *piperidine*,



is a secondary base; coniine and sarcosine (methylglycine) are also secondary bases.

When one hydrogen atom of ammonia is replaced by an acid radical, such as acetyl or benzoyl, an *amide* is obtained, *e. g.*, acetamide, $CH_3.CO.NH_2$. Mixed compounds, such as methylacetamide, $NHMe.CO.CH_3$, and acetanilide, $NHPh.CO.CH_3$, are formed by the replacement of one hydrogen atom of ammonia by hydrocarbon radical and one by an acid radical. Urea or carbamide see vol. [VII] is the diamide of carbonic acid, and has the constitution $NH_2.CO.NH_2$.[1] Guanidine vol. [VII] is the corresponding *imine*, $NH:C(NH_2)_2$.

Of these various compounds, the monamines may with advantage be considered at the present stage, but the majority of the amines will be dealt with in other sections.

MONAMINES

These bases are derived from 1 molecule of ammonia by the substitution of 1 or more of the hydrogen atoms by an equivalent num-

[1] This formula for area is strongly opposed by E. A. Werner who as the result of many investigations gives it a cyclic structure $NH.C\begin{cases} NH_3 \\ O \end{cases}$. See *J. Chem. Soc.*, 1914, 105, 924; 1915; 715; 1916, 1120; and other papers.

ber of alkyl radicals. The lower members are strongly basic in character and have an ammoniacal odour, the solubility, basicity and volatility tend to decrease with increasing molecular weight. The first substance obtained of this class was ethylamine, $C_2H_5.NH_2$, prepared by Wurtz in 1848 by distilling ethyl cyanurate with potassium hydroxide. Methylamine, $CH_3.NH_2$, was obtained by the same chemist in the following year, by the distillation of methyl isocyanate with alkali hydroxide: $2KOH + CH_3.N.CO = K_2CO_3 + CH_3.NH_2$.

Hofmann obtained the monamines by the action of an alkyl iodide on an alcoholic solution of ammonia. The action is not a simple one, all three monamines being formed, together with a tetra-alkyl-ammonium base. Thus, when ethyl iodide is heated with alcoholic ammonia to 100° in a sealed tube, there are obtained:

Hydriodide of ammonia,	H_3N,HI.
Hydriodide of ethylamine,	$(C_2H_5)H_2N,HI$.
Hydriodide of diethylamine,	$(C_2H_5)_2NH,HI$.
Hydriodide of triethylamine,	$(C_2H_5)_3N,HI$.
Tetra-ethyl-ammonium iodide,	$(C_2H_5)_3N,C_2H_5I$.

Similar products result when ethyl bromide or chloride is substituted for the iodide, except as to the relative proportions of the amines obtained. Thus ethyl chloride produces almost exclusively $EtNH_2,HCl$, with small quantities of Et_2NH,HCl and $NEt_3,EtCl$; ethyl bromide gives chiefly $EtNH_2,HBr$ with very appreciable quantities of $NHEt_2HBr$ and NEt_3,HBr, but very little NEt_4Br; while ethyl iodide produces NH_2Et,HI, $NHEt_2,HI$, and NEt_3,HI in about equal proportions, as well as very appreciable quantities of Et_4NI (Groves, *J. Chem. Soc.*, **13**, 331).

A similar series of products is obtained by heating methyl iodide, bromide, or nitrate with a solution of ammonia in methyl alcohol. When the methyl nitrate and ammonia solution are used in equivalent proportions for the action—$MeNO_3 + H_3N = NH_2Me,HNO_3$, methylamine is the chief product, though more or less of each of the more highly substituted products is also formed. With excess of methyl nitrate, tetramethyl-ammonium nitrate, $Me_4N.NO_3$, is produced in large excess, and the same quarternary compound is formed if methyl bromide or iodide be substituted for the nitrate.

The complex nature of the products obtained by treating alkyl iodides, etc., with alcoholic ammonia is due to the tendency of the

amines first produced to act on the remaining portions of the alkyl iodide or other salt to form ammonium iodide and more highly substituted amines. The hydriodides of the amines similarly react with alkyl iodides in presence of ammonia to form ammonium iodide and more highly substituted amines.

From these reactions it follows that diethylamine hydriodide, for instance, may be obtained by heating ethyl bromide or iodide with a calculated amount of ethylamine in a sealed tube. A great variety of mixed amines may be obtained by similar means.

There are many other methods of preparing the amines, among which may be mentioned the catalytic reduction processes of Sabatier and Senderens which forms primary aliphatic amines from nitriles or nitro-compounds at 180°–200° and aromatic amines from aromatic nitro compounds at 150°–180°.

Separation of Amines from Tetralkylammonium Salts

In the preparation of an amine by means of the alkyl halides a mixture of the salts of primary, secondary and tertiary bases is obtained together with the quaternary ammonium salt. The product of the action is filtered from ammonium iodide, which is nearly insoluble in the alcoholic liquid, and is evaporated to dryness to remove excess of alcohol, free ammonia, and unchanged alkyl iodide. The residue is then distilled with potassium hydroxide, when the hydriodides of the amines are decomposed, the bases volatilising, while the tetra-alkyl ammonium iodide remains in the retort unchanged by, and insoluble in, the strong potassium hydroxide solution. The mixture of amines is conducted over calcium oxide, and then condensed by passage through a well-cooled tube.

Detection and Separation of Primary, Secondary and Tertiary Amines

In addition to a number of methods applicable to the separation of particular amines, there are four general methods which, with slight variation, are available for most mixtures of primary, secondary and tertiary amines. These are: Hinsberg's method using sulphochlorides, acetylation, Hofmann's original oxalate process, and by the use of a Grignard reagent.

1. **Hinsberg's Method.**—A useful method of separating or distinguishing the three classes of amines is that of Hinsberg (*Ber.*,

1890, **23**, 2962, and *Annalen*, 1891, **265**, 178). It depends on the fact that whereas tertiary amines are not affected by aromatic sulphochlorides, such as benzenesulphochloride, primary and secondary amines yield sulphonamides, *e. g.*, $C_6H_5.SO_2.NHR$ and $C_6H_5.SO_2.$-NR_1R_2, when shaken with the sulphochloride in presence of alkali. The sulphonamides of the first type, moreover, differ from those of the type $C_6H_5.SO_2.NR_1R_2$, in forming alkali salts which are soluble in water. After the action of the sulphochloride is completed the excess of alkali is nearly neutralised and the product subjected to steam distillation which generally serves to remove the tertiary base. The sulphonamide of the primary base can then be separated from that of the secondary base by taking advantage of its solubility in aqueous alkali, the sulphonamide of the secondary amine remaining undissolved. The amines are regenerated from their sulphonamides, after separation, by heating the latter with hydrochloric or sulphuric acid in a sealed tube at 130–150° (Hinsberg); or by warming with chlorosulphonic acid, SO_3HCl, in an open vessel at 130–150°, followed by boiling with dilute alkali (Marckwald and von Droste-Huelshoff, *Ber.*, 1898, **31**, 3261). In some cases the action is not strictly normal, alkali-insoluble dibenzene-sulphonyl compounds, $RN(SO_2.C_6H_5)_2$, being formed from the primary amine along with the normal alkali-soluble monobenzenesulphonyl derivative, thus causing confusion. In the case of primary amines with more than 6 carbon atoms, as well as with certain amino-compounds of the terpene series, the sodium compounds of the true monobenzenesulphonamides are easily hydrolysed by water and insoluble in an excess of alkali, and a false conclusion as to the presence of secondary bases may thus be arrived at. According to Hinsberg and Kessler (*Ber.*, 1905, **38**, 906), however, these difficulties may be overcome by the following procedure: 1. The abnormal dibenzene-sulphonyl compounds can be hydrolysed to the normal alkali-soluble forms by warming with sodium ethoxide dissolved in alcohol. 2. The abnormal insoluble monobenzenesulphonamides can be converted by sodium in ethereal solution into sodium salts which are *insoluble* in ether, while the sulphonamide-derivatives of secondary bases are without exception soluble in ether and unaffected by sodium.

2. **Acetylation.**—Primary and secondary amines are readily attacked, generally at the ordinary temperature, more rapidly on warming, by acetyl chloride or acetic anhydride (a sufficiency to wet

the compound thoroughly, generally 1.5 to 5 times the theoretical quantity required for acetylation). Primary bases form acetyl derivatives of the type RNHAc, and secondary bases compounds of the type R_1R_2.NAc. Tertiary bases do not form acetyl derivatives, but, when treated with acetyl chloride or acetic anhydride and subsequently with water, dissolve in the form of chloride or acetate respectively. Thus, on acetylating a mixture of a primary, secondary and tertiary amine, as the acetyl derivatives formed from the first two are as a rule sparingly soluble in water, on diluting the reaction product with a large volume of water, the tertiary base dissolves and leaves the acetyl derivatives undissolved; on adding alkali hydroxide to the solution of the soluble salt of the tertiary amine, the free base is separated and can be suitably dealt with. The mixture of acetyl compounds is hydrolysed by heating with concentrated hydrochloric acid and the secondary base separated from the primary amine in the form of nitrosamine (see page 277). The primary base is converted by this treatment into the corresponding alcohol.

3. **Hofmann's Method.**—(*a*) If an amine be heated to 100°, under pressure, with an excess of alkyl iodide, a quaternary iodide will at length be formed, and the problem whether the original base was a primary, secondary, or tertiary amine will be solved by comparing the composition of the ultimate product with that of the original base or its hydriodide. Thus, if methyl iodide has been the alkylating agent employed, the iodide ultimately obtained will differ from the hydriodide of the original base by $3CH_2$ if the amine was primary; by $2CH_2$ if secondary, and by CH_2 if tertiary.

(*b*) The following is an outline of the method devised by Hofmann for the separation of mixed ethylamines.

The bases are treated in a flask with one and a half times their weight of ethyl oxalate (previously dried over calcium chloride), which is added gradually through a tap funnel. This has no action on triethylamine or other tertiary bases, but converts diethylamine into liquid ethyl diethyloxamate, and ethylamine into solid diethyloxamide,[1] according to the following equations:

[1] Diethyloxamide may also be separated from the ethyl diethyloxamate by cold water in which the former dissolves easily, the latter very sparingly. If hot water be used, the separation is more perfect and the residual oxamate quite pure; but some of it suffers hydrolysis and goes into solution as diethyloxamic acid.

$$1.\quad \begin{array}{l} CO_2Et \\ | \\ CO_2.Et \end{array} + 2EtNH_2 = \begin{array}{l} CO.NHEt \\ | \\ CO.NHEt \end{array} + 2EtOH$$

Ethyl oxalate — Ethylamine — Diethyloxamide — Alcohol

$$2.\quad \begin{array}{l} CO.OEt \\ | \\ CO.OEt \end{array} + Et_2NH = \begin{array}{l} CO.OEt \\ | \\ CO.NEt_2 \end{array} + EtOH$$

Ethyl oxalate — Diethyl-amine — Ethyl diethyl-oxamate — Alcohol

The liquid gets very hot, but for the completion of the action the mixture should be heated to 100° for several days in a closed vessel. The triethylamine, which has taken no part in the change, is then distilled off on the water-bath. The residue is well cooled, and the solid oxamide separated from the liquid oxamate by pressure.[1] On subsequent distillation with potassium hydroxide these compounds yield the primary and secondary amines respectively:

$$1.\quad C_2O_2(NH.Et)_2 + 2H.(OK) = C_2O_2(OK)_2 + 2H(NH.Et)$$

$$2.\quad \begin{array}{l} CO.OEt \\ | \\ CO.NEt_2 \end{array} + 2HOK = \begin{array}{l} CO.OK \\ | \\ CO.OK \end{array} + NHEt_2 + HOEt.$$

The foregoing process, with certain modifications in detail, is of general application for the separation of primary, secondary, and tertiary amines; the first class forming oxamides, the second oxamic esters, and the third being unaffected.

An important modification in the foregoing method has been made by Duvillier and Buisine (*Ann. Chim. Phys.* [v], **23**, 289) who operated on an aqueous solution of the bases. Under these conditions, the primary amines are converted by ethyl oxalate into insoluble or sparingly soluble oxamides, while the secondary and tertiary bases are unchanged, or at any rate remain wholly in solution. After separating the oxamides by filtration, the mother-liquor[2] [is boiled for some time, which causes the hydrolysis of the ethyl diethyloxamate with formation of diethyloxamic acid, $\begin{array}{l} CO.OH \\ | \\ CO.NEt_2, \end{array}$ and the further change of this into the acid oxalate of diethylamine, $(C_2H_5)_2HN.H_2C_2O_4$. This

[1] Some ethyl monoethyloxamate, $C_2O_2 \left\{ \begin{array}{l} O.C_2H_5 \\ NH.C_2H_5 \end{array} \right.$, is always formed from the primary amines in this reaction.

[2] The treatment described in the brackets is optional, and chiefly of advantage in the separation of ethylamines.

salt separates on cooling, and yields the free base on distillation with alkali. The filtrate] is distilled with potassium hydroxide, the bases dried by potassium hydroxide, and dissolved in absolute alcohol. On adding ethyl oxalate to this solution the secondary amines are converted into oxamic esters, while any remaining primary amines are converted into the corresponding oxamides. After allowing the mixture to stand for 24 hours to complete the action, the alcohol and unchanged *tertiary bases* are distilled off on the water-bath. The oxamates remaining in the retort may be converted into calcium salts by treatment with milk of lime, or the *secondary bases* may be at once liberated and recovered by distillation with potassium hydroxide.[1]

Duvillier and Buisine have applied this method to the analysis of the complex mixture of amines present in commercial trimethylamine from *vinasses* (page 285). A. Müller (*Bull. Soc. Chim.*, 1884, **42**, 202) has described a method for the separation of amines based on much the same principle.

4. By Grignard's reagents. Hibbert and Wise (*J. Chem. Soc.*, 1912, **101**, 344) point out how Grignard's reagent serve as a general reagent for the separation of amines, particularly those of the aromatic series. In the case of primary and secondary amines the additive compounds formed with the magnesium alkylhalides are extremely unstable at the ordinary temperature, decomposing practically instantaneously with the evolution of the corresponding fatty hydrocarbon:

$$R.NH_2 + MgR'I = R.NH(MgI) + R'H \qquad (1)$$
$$R:NH + MgR'I = R_2:N(MgI) + R'H \qquad (2)$$

Whilst tertiary amines, not possessing any available hydrogen atom, yield additive compounds incapable of decomposing in this manner. To a mixture of the amines dissolved in ether is added an excess of an ether solution of magnesium ethyl bromide, (the Grignard reagent is added until no further evolution of gas occurs) then the ether is distilled. The resulting product is heated in an oil bath to a temperature between 200° and 280° according to nature of the amine

[1] The conversion into calcium salts is especially suitable for the treatment of the ethylamines. The precipitated calcium diethyloxamate and monoethyloxamate are filtered off, and the filtrate treated with alcohol, which precipitates the remainder of the calcium salts. The precipitates are treated with boiling water, when the monoethyloxamate dissolves, and is deposited again on cooling in large crystals, which on distillation with potassium hydroxide yield *ethylamine*. On concentrating and cooling the mother-liquors, calcium diethyloxamate separates. It is recrystallised from alcohol, washed with ether to remove any adhering oxamide, and distilled with potassium hydroxide, when it yields pure *diethylamine*.

(amyl or butyl amines require a temperature of about 245°), whereupon the tertiary amine is distilled over. The bulk of secondary or primary amine may be recovered by adding sodium hydroxide to the residue and distilled in a current of steam. The method is available for the estimation of amines see p. (278).

Price (*J. Soc. Chem. Ind.*, 1918, **37**, 82T) gives a method particularly applicable to the separation of secondary amylamines from primary amines by means of sulphuric acid.

Qualitative Reactions

The primary, secondary, and tertiary monamines may also be distinguished by the following reactions.

5. If a primary monamine be boiled with alcoholic potassium hydroxide and chloroform, the characteristic and highly disagreeable odour of the corresponding carbamine or isonitrile is evolved, according tothe equation: $MeNH_2 + CHCl_3 + 3KHO = MeNC + 3H_2O + 3KCl$.

6. If a primary monamine be dissolved in a mixture of equal volumes of alcohol and carbon disulphide, and the liquid then boiled down to one-half, a thiocarbamate will be formed thus: $2MeNH_2 + CS_2 = MeNH.CS.S.HNH_2Me$.

If the resultant liquid be boiled with a solution of mercuric or ferric chloride, a pungent odour of mustard oil will be produced owing to the formation of an alkyl iso-thiocyanate:[1]

$$MeNH.CS.S.NMeH_3 + HgCl_2 = HgS + MeNCS + NMeH_3Cl + HCl.$$

Secondary amines combine with carbon disulphide under the same conditions, but give alkylthiocarbamic acids which are not convertible into a "mustard-oil."

$$CS_2 + 2NHEt_2 = NEt_2.CS.SH,NHEt_2.$$

7. Nitrous acid converts *primary fatty monamines* into the corresponding alcohols: $MeH_2N + NO.OH = Me.OH + OH_2 + N_2$.

Aromatic amino-compounds (*e. g.*, aniline) are converted by nitrous acid into diazo-compounds: $PhNH_2 + NO.OH + HCl = Ph.N{:}NCl + 2H_2O$, which on boiling with water yield phenols.

Secondary amines, whether fatty or aromatic, are converted by nitrous acid into nitrosamines, thus: $Me_2NH + NO.OH = Me_2N.NO + H_2O$. The nitrosamines are yellow liquids, of neutral character

[1] In the case of aromatic primary amines, the product is usually a thio-urea, which requires to be treated with phosphorus pentoxide to obtain the iso-thiocyanate.

and aromatic odour, volatile without decomposition in a current of steam. Weak reducing agents (zinc dust and acetic acid) convert them into hydrazines; but by more powerful hydrogenising agents, or by warming with alcohol and hydrochloric acid, they are reconverted into the original secondary amines.

These nitrosamines give an intensely blue or bluish-violet coloration when they are warmed with phenol and concentrated sulphuric acid and the mixture diluted with water (*Liebermann's nitroso-reaction*).

Nitrous acid has no action on *tertiary fatty amines.* It converts most *tertiary aromatic amines* into nitroso-derivatives which contain the nitroso-group in the benzene nucleus.

In practice, the action of nitrous acid on the amines is best effected by distilling their hydrochlorides with a strong solution of potassium or sodium nitrite after adding the necessary quantity of hydrochloric acid. If a mixture of the 3 methylamines be thus treated, the *methylamine* is destroyed (with formation of methyl alcohol, which will be found in the distillate), *dimethylamine* is converted into dimethyl-nitrosamine, which distils,[1] while the hydrochloride of *trimethylamine* remains in the retort, and on distilling it with alkali hydroxide the free base can be obtained.

This method, however, loses its quantitative value owing to the fact that a portion of the tertiary amine may undergo conversion into the secondary amine by an alkyl group being split off in the form of aldehyde; the amount of nitrosamine is thus increased.

8. The acid ferrocyanides of the *tertiary amines* are remarkably insoluble in water. They are precipitated on adding potassium ferrocyanide to the solutions of the amines acidified with hydrochloric acid. The bases can be recovered from their ferrocyanides by treating the precipitate with solution of cupric sulphate, filtering, and removing the sulphuric acid and excess of copper from the filtrate by barium hydroxide. (Fischer, *Annalen*, 1878, **190**, 185; Chrétien, *Compt. Rend.*, 1902, **135**, 901.)

Estimation of Amines.—The application of the Grignard reagent, to which reference has already been made, p. (276), affords a method of estimation which is available for the estimation of primary, secondary and tertiary amines. At ordinary temperatures one mole-

[1] On separating the nitrosamine, which forms a yellow oil, from the aqueous distillate, treating it with aqueous hydrochloric acid, and then passing hydrochloric acid gas till the liquid is homogeneous, the hydrochloride of the secondary amine is formed, and may be obtained by evaporation of the solution.

cule of the amine, whether primary or secondary, liberates one molecule of methane from methyl magnesium iodide; when the temperature is raised to 120°–125° no change occurs in the case of a secondary amine, but the magnesium derivative of the primary amine reacts with excess of the Grignard reagent, evolving a second molecule of methane. Tertiary amines evolve no gas; hence primary and secondary amines can be estimated in the presence of the tertiary compounds. (Sudborough and Hibbert, *J. Chem. Soc.*, 1909, **105**, 477.) For the preparation of the Grignard compound, 6.09 grm. of dry magnesium turnings are added to 120 c.c. of dry phenetole, and 35.5 of methyl iodide diluted with 20 c.c. of phenetole are introduced. The mixture is heated on a sand bath to initiate the reaction; after the magnesium has all disappeared the mixture is heated to the b. p. to expel excess of methyl iodide, then, after cooling, the clear solution is made up to 175 c.c. with phenetole. The reagent must be carefully kept from contact with moisture. For the estimation a stout flask of about 200 c.c. capacity is fitted with a rubber stopper carrying an outlet and inlet tube with taps; the inlet tube should reach nearly to the bottom and is used to displace the air in the flask by nitrogen (although this precaution is not always necessary). About 0.2 grm. of the amine is mixed with 15 c.c. of phenetole in the flask, then a similar volume of the solution of the Grignard compound is introduced in a small tube which is lowered vertically into the flask. The whole is connected with a nitrometer filled with mercury, and when the air has been expelled the reagents are allowed to mix, and the volume of methane evolved at the ordinary temperature is noted, together with the temperature and barometric pressure. The mixture is now heated to 120°–125° for 45 minutes and, after cooling, the additional volume of gas is measured. From the stoichiometric equations already given it is easy to calculate the quantity of amine present.

Both *primary* and *secondary monamines* react with aldehydes to form neutral compounds. The action between œnanthal and mono- and di-methylamine respectively is as follows:

$$H_2.N.CH_3 + C_6H_{13}.CHO = H_2O + C_6H_{13}.CH.NCH_3; \text{ and}$$
$$2HN(CH_3)_2 + C_6H_{13}.CHO = H_2O + C_6H_{13}.CH[N(CH_3)_2]_2.$$

This reaction has been utilised by Schiff (*Annalen*, 1871, **159**, 158) for the volumetric assay of amines. The base is dissolved in benzene,

fused calcium chloride added, and then a standard solution of œnanthal in benzene dropped in from a burette so long as water continues to separate. Each addition of the œnanthal solution produces a turbidity from separation of water, but this is absorbed by the calcium chloride on gentle agitation. As a primary amine reacts with twice as much œnanthal as the corresponding secondary amine, the proportions of the two in a mixture can be estimated from the result of the titration, provided the mean combining weight of the mixture be known, or ascertained in a separate experiment by titration with standard acid.

Generic Characters of Monamines

The monamines, as a class, are readily volatile liquids, of lower sp. gr. than water. Their b. p. rise with the number of carbon atoms in the molecule. The lower members dissolve with great facility in water, forming strongly alkaline liquids of an ammoniacal odour. The higher members are, however, without odour and do not dissolve in water. From their solutions, ethylamine and the higher homologues can be separated by saturating the liquid with potassium hydroxide. By boiling the aqueous solutions of the free bases, or of their salts after adding excess of lime or alkali hydroxide, the monamines can be completely volatilised, and condensed again in water or acid, and titrated in the same manner as ammonia. The monamines are all powerful bases, closely resembling ammonia in their general characters. They are, however, distinguished from ammonia by their inflammability, a fact which led to their discovery; they burn with a yellow flame. They form crystallisable salts and well defined double-salts, such as the *aurichlorides* and *platinichlorides*, which are very useful for their identification and analysis; the *picrates* are also generally well defined. The monamines precipitate magnesium salts, but the precipitated magnesium hydroxide dissolves in the amine hydrochloride, forming a double salt from the solution of which phosphate of sodium precipitates an amino-magnesium phosphate. The amines thus behave exactly in the same manner as ammonia.

The only amines (not described in other chapters) requiring detailed consideration are the primary, secondary, and tertiary monamines of methyl and ethyl. These substances are typical of the amines generally, and most of the statements made respecting them would be true of all the compounds of this class. Their aqueous

solutions dissolve silver chloride, and behave in much the same manner as ammonia with metallic salts; but there are some interesting differences, as shown in the table below, from which is will be seen that certain of the precipitates which are soluble in excess of ammonia are undissolved by the amines, and *vice versa*.[1]

Metallic salt	Ammonia H_3N	Ethylamine $(C_2H_5)H_2N$	Methylamine $(CH_3)H_2N$	Dimethylamine $(CH_3)_2HN$	Trimethylamine $(CH_3)_3N$
Aluminium ...	Insoluble (nearly).	Soluble.......	Soluble.......	Soluble.......	Soluble..... .
Cobalt.......	Blue precipitate; soluble in excess to brown solution.	Insoluble.....	Blue; insoluble in excess; turned brownish on heating.	Blue; insoluble in excess; turned brownish on heating.	Blue; insoluble in excess; turned brownish on heating.
Nickel........	Soluble in excess to violet-blue solution.	Insoluble.....	Apple-green; insoluble in excess.	Apple-green: insoluble in excess.	Apple-green: insoluble in excess.
Zinc........ .	Very soluble.	Soluble.......	Soluble in large excess; reppted on heating.	Soluble in large excess; reppted on heating.	Soluble in very large excess; reppted on heating.
Cadmium... .	Soluble......	Insoluble.....	Insoluble.. ..	Insoluble.....	Insoluble.
Silver........	Brownish; very soluble in excess.	....	Brownish; soluble in large excess; reppted on warming.	Brownish; soluble in large excess; reppted on warming.	Dirty brown ppt. changing to black; sol. large excess to dark solution; reppted on warming.
Cupric.......	Blue; soluble in excess to deep blue solution.	Soluble with difficulty in excess.	Blue; soluble in large excess to deep blue solution; reppted dirty brown on boiling.	Blue; partly soluble in large excess; reppted dirty brown on boiling.	Blue; partly soluble in large excess; reppted dirty brown on boiling.
Mercuric.....	White.......	....	White; insoluble.	White; soluble in much water.	Yellow; changing to very pale yellow.
Stannic.......	Insoluble....	Very soluble in excess.	. .. . .	Soluble..... .	Soluble.
Antimonic....	....	....	. . . .	Soluble.....	Soluble in large excess.
Gold.........	Insoluble....	Soluble.......	Brownish yellow ppt., readily soluble in excess to orange-red liquid.	Yellow precipitate; soluble in excess to brown liquid.	. . . .
Ruthenium ..	Insoluble....	Soluble....	. . . .	. . . . .	. . .
Lead.......	Insoluble...	.. . .	Insoluble..	Insoluble....	Insoluble.

[1] Allen was indebted to Leo Taylor for repeating and enlarging on the experiments of Vincent, on whose observations the table is chiefly founded. Several blanks in the observations of Vincent have been filled by Taylor.

In all cases a solution of aluminium phosphate in hydrochloric acid behaves similarly to a solution of aluminium chloride (Taylor)

PHYSICAL PROPERTIES OF AMINES

The following table is taken from Meyer-Jacobson's *Lehrbuch* (1906)

Alkyl radical R	Primary amine NH_2R			Secondary amine NHR_2		Tertiary amine NR_3	
	M. p.	B. p.	Sp. gr.	B. p.	Sp. gr.	B. p.	Sp. gr.
Methyl	...	−6.7°	0.699(−11°)	+7°	0.686(−6°)	+3.5°	0.662(−5°)
Ethyl	−83.8	+19	0.708(−2°)	56°	0.711(+15°)	90°	0.735(+15°)
Propyl	...	49°	0.728(0°)	110°	0.738(20°)	156°	0.771(0°)
iso-Propyl	...	32°	0.690(18°)	84°	0.724(15°)	...	...
n-Butyl	...	77.8°	0.742(15°)	160°	...	216.5°	0.791(0°)
iso-Butyl	...	66°	0.735(15°)	136°	...	187°	0.785(21°)
sec-Butyl	...	63°	0.718(20°)		...	...	...
tert-Butyl	...	43.8°	0.698(15°)		...	...	...
n-Amyl	...	104°	0.766(19°)			...	...
iso-Amyl	...	95°	0.750(18°)	187°	0.782(0°)	235°	...
tert-Butyl methyl	...	82–83°	...	...	...	...	...
sec-*n*-Amyl (Methyl-n-propyl-carbinamine)	...	92°	0.738(20°)			...	...
sec-*n*-Amyl (Diethylcarbinamine)	...	90–91°	0.749(20°)		...		...
Methyl *iso*-propyl carbinamine	...	83–84°	0.757(18.5°)		...		
tert-Amyl (Dimethylethyl carbinamine)	...	78.5°	0.748(15°)	...		...	...
n-Hexyl	...	129°	...	...	...	260°	...
n-Heptyl	...	153°	0.777(20°)	...	...	...	...
n-Octyl	...	175–177°	0.777(26.8°)	297°	...	366°	...
n-Nonyl	...	190–192°	...			...	
n-Decyl	+17	216–218°	...	...	...	...	...

Methylamine, $NH_2.CH_3$

Methylamine exists ready-formed in *Mercurialis annua* and *M. perennis*, and, as obtained (in an impure state) from these plants, was formerly known as mercurialine. It also exists in herring-brine, coal

tar, bone-oil, and the products of the distillation of wood, beetroot molasses (*vinasses*), and certain alkaloids (*e. g.*, morphine, codeine). It is also produced when caffeine is boiled with baryta-water, and by heating trimethylamine hydrochloride to 285°, when methyl chloride and trimethylamine volatilise, and methylamine hydrochloride (mixed with some ammonium chloride) remains. Methylamine is prepared commercially by electrolysis of a solution of hexamethylene tetramine and ammonium sulphate, lead electrodes in a cell having a porous diaphragm being used; the products methylamine and trimethylamine are separated by Hofmann's method. In the laboratory it may be obtained pure by treating one equivalent of acetamide with 2 equivalents of bromine, and then adding a 10% solution of potassium hydroxide till the colour of the bromine has nearly disappeared:

$$C_2H_3O.NH_2 + Br_2 + 2KHO = C_2H_3O.N.BrK + KBr + 2H_2O.$$

Three additional equivalents of potassium hydroxide are now dissolved to form a 10% solution, and heated in a retort to 70°. The product of the first action is then gradually added through the tubulure. The gases evolved are collected in hydrochloric acid, and on evaporating the solution a mixture of the hydrochlorides of ammonia and methylamine is obtained,[1] from which the latter only is dissolved by absolute alcohol.[2] On distillation with alkali hydroxide or slaked lime the salt yields the base, quite free from di- or tri-methylamine.

Methylamine boils at −6.7°, and hence is a gas at ordinary temperature. 1 volume of water at 12.5° dissolves 1150 volumes of the gas, and hence it is more soluble even than ammonia, which methylamine closely resembles in odour and general characters; methylamine is distinguished by its ready inflammability—a property even possessed by its concentrated aqueous solution. It burns with a yellow flame, forming carbon dioxide water, nitrogen, and hydrocyanic acid.

On passing a succession of electric sparks through methylamine, hydrocyanide of methylamine is produced, and this is decomposed by a continuation of the treatment, with formation of a tarry deposit. When passed through a red-hot tube, methylamine is decomposed, with formation of hydrogen and ammonium cyanides, methane, and hydrogen.

[1] The reaction which occurs is very complex (A. W. Hofmann, *Ber.*, 1882, **15**, 765), but the main decomposition may be expressed as follows:

$$CH_3.CO.NKBr + 2KOH = CO(OK)_2 + KBr + CH_3.NH_2.$$

[2] See also H. Quantin, *Ann. Chim. Anal.*, 1906, **6**, 125.

Methylammonium chloride, NH_2Me,HCl, melts at 225–226°. *Methylammonium picrate*, $NH_2Me,C_6H_3O_7N_3$, melts at 215°. The *platinichloride*, $(MeH_3N)_2PtCl_6$, is insoluble in alcohol, but soluble in boiling water, crystallising, on cooling, in beautiful golden-yellow scales.

A convenient method for the differentiation of methylamine and ammonia is based by Valton (*J. Chem. Soc.*, 1925, **127**, 40) upon the greater reactivity of the former with 2:4 dinitrochlorobenzene. This affords a method of detecting small quantities of methylamine in the presence of ammonia; dimethylamine must not be present in amount exceeding 10 per cent. of the methylamine. The solution is distilled, in a flask fitted with a splash trap, with 30 c.c. of 2N sodium hydroxide and about 80 c.c. of water into 10 c.c. of 0.5 per cent. alcoholic solution of 2:4 dinitrochlorobenzene. When 10 c.c. have passed over the distillation is stopped, and the solution is left for 24 hours. The precipitated dinitromethylaniline is crystallised once more from alcohol and identified by the method of mixed melting points; dinitromethylaniline so prepared melts between 170° and 175°.

A method for the proximate analysis of the bases present in crude methylamine, based on the principles of the process described on page 275, has been described by A. Müller (*Bull. Soc. Chim.*, 1884, **42**, 202).

Dimethylamine, $NH(CH_3)_2$

Dimethylamine occurs in Peruvian guano and pyroligneous acid, and is also present in the products of the distillation of *vinasses*. It is formed when fish undergo putrefaction. It often forms a large percentage of commercial "trimethylamine." It is best separated from methylamine and trimethylamine by the action of nitrous acid, but, as already pointed out (page 278), a small proportion of trimethylamine is converted by this treatment into the secondary base. Dimethylamine may also be obtained pure by boiling 35 parts of nitroso-dimethylaniline hydrochloride with a solution of 15 parts of potassium hydroxide in 400 of water:

$$C_6H_4(NO).NMe_2,HCl + KHO = KCl + C_6H_4(NO).OH + NHMe_2.$$

Dimethylamine boils at 7°, has a sp. gr. 0.686 at −6°, and closely resembles the primary and tertiary methylamines. From the former

it is at once distinguished by the non-formation of a precipitate on the addition of ethyl oxalate to the aqueous solution of the base (page 275), and the non-production of an isonitrile on treatment with alcoholic potassium hydroxide and chloroform. From trimethylamine it is distinguished by the formation of a nitrosamine on treating it with nitrous acid. It gives a white precipitate with Nessler's reagent.

The *platinichloride*, $(Me_2H_2N)_2PtCl_6$, crystallises in very long needles.

Dimethylnitrosamine boils at 149°.

Dimethylamine hydrochloride is remarkable in being soluble in chloroform, a fact which permits of its ready separation from ammonium chloride, which is insoluble in this solvent.

Trimethylamine, $N(CH_3)_3$

Trimethylamine is found notably in herring-brine, and has been detected in urine, unputrefied blood of the calf, cod-liver oil, and other animal fluids. It occurs in the *Chenopodium vulvaria* (stinking goose-foot), from the leaves of which it constantly exudes; *Arnica montana; Mercurialis annua;* the blossoms of the pear, white-thorn (*Cratægus oxyacantha*), hawthorn, and wild cherry; and in ergot and other parasites of the vegetable kingdom. Trimethylamine is also a product of the dry distillation of certain alkaloids, wood, etc., but especially of the *vinasses* or residue left after the distillation of the spirit from fermented beet-root molasses. The bases obtained by the destructive distillation of this product are derived from the betaine.

The products of the destructive distillation of the "vinasses,"[1] left after the distillation of the fermented beetroot-molasses, vary with the concentration of the liquid. As the proportion of water

[1] The *vinasses*, or spent wash from the stills, is evaporated till it acquires a sp. gr. of 1.31, when it is subjected to dry distillation in cast-iron retorts. The aqueous portion of the distillate contains: Ammonium carbonate, sulphydrate and cyanide; methyl alcohol, methyl sulphide, and methyl cyanide; various other substances of the fatty series; and a large proportion of salts of trimethylamine. The tar yields, on distillation: ammoniacal liquor, various oils, pyridine bases, solid hydrocarbons, phenols, and pitch of superior quality. The aqueous liquid is neutralised with sulphuric acid and concentrated, when crystals of ammonium sulphate are deposited, and vapours of methyl alcohol are evolved together with methyl cyanide and other nitriles. The methyl cyanide is converted into ammonia and acetate by treatment with an alkali: $CH_3.NC + NaHO + H_2O = H_3N + CH_3.COONa$. The dark-coloured mother-liquors retain the trimethylamine sulphate, which is decomposed by distillation with lime, the vapours being passed into hydrochloric acid. The resulting solution is boiled down till the temperature reaches 140°. Ammonium chloride crystallises out on cooling, and the mother-liquor is separated and concentrated till the b. p. rises to 200°, the product forming commercial trimethylamine hydrochloride, from which the free base may readily be obtained by treatment with lime or alkali hydroxide.

decreases, the quantity of ammonia increases, and the trimethylamine is replaced by the primary and secondary methylamines. The *vinasses* from different localities yield varying proportions of gaseous and liquid products on distillation, the nitriles and methyl alcohol appearing to be the most variable constituents.[1]

Pure trimethylamine may be prepared in the laboratory by heating ammonium chloride (50 grm.) with 40% formaldehyde solution (440 grm.) at 120° in an autoclave. The action is finished when the internal pressure has reached a value of 35–40 atms. (Eschweiler and Naeppen, *Ber.*, 1905, **38**, 882).

A more convenient way, starting from commercial trimethylamine, is to add to the hydrochloride in a large flask excess of strong solution of sodium hydroxide; the mixture is distilled and the impure base

[1] In a specimen of "commercial trimethylamine," prepared from vinasses, Duvillier and Buisine found only from 5 to 10% of trimethylamine and some 50% of dimethylamine; while the remainder consisted of methylamine, propylamine, and isobutylamine in about equal proportions; the ethylamine being estimated at about 2%, and ammonia being absent (*Compt. Rend.*, 1879, **89**, 48). The method employed by these chemists for the separation of the amines in question was as follows (*Ann. Chim. Phys.* 1881, **23**, 289): The aqueous solution of the free bases was treated with ethyl oxalate, the dense white precipitate of oxamides filtered off, the filtrate concentrated by distillation, and the further precipitate added to that previously obtained. By treating the precipitate with hot water it was separated into 3 fractions. The most insoluble portion 1. consisted of dibutyloxamide (or possibly di-*iso*-butyloxamide), which melted and floated on the hot water, and on cooling formed a solid waxy mass. When recrystallised from alcohol, it was obtained in pearly needles. The ***butylamine***, obtained by distilling the oxamide with potassium hydroxide, had a faintly aromatic odour, and yielded a slightly soluble platinichloride, crystallising in orange-coloured plates. Of the oxamides soluble in boiling water, the dipropyl compound 2. was first deposited. It crystallised from alcohol in pearly needles melting at 110°, and the ***propylamine***, obtained from it gave an orange platinichloride. When the proportion of butylamine and propylamine was small, the authors preferred to utilise the comparative insolubility of their sulphates in alcohol to separate them from the other amines. The most soluble portion of the mixed oxamides 3. was deposited in opaque white needles or grains, and consisted of dimethyloxamide. The base obtained by distilling it with potassium hydroxide was converted into the sulphate, which on treatment with boiling absolute alcohol was obtained quite pure, and yielded pure ***methylamine*** on treatment with potassium hydroxide.

The mother-liquor separated from the oxamides of the primary amines was distilled with potassium hydroxide, and the dried gas collected in absolute alcohol. A portion of the solution was then titrated with standard acid, and the remainder gradually added to a quantity of ethyl oxalate sufficient for the action: $Me_2NH + Et_2C_2O_4 = (MeHN)_2C_2O_2 + 2EtOH$; assuming the alkalinity to be wholly due to dimethylamine. The operation was conducted in a flask, which was surrounded with ice and continually shaken. When the action was completed, the flask was heated on the water-bath, and the alcohol and unchanged ***trimethylamine*** distilled off and collected in hydrochloric acid. It yielded a platinichloride in large orange-red crystals, and was the only tertiary amine found in the mixture of bases under examination.

The syrupy residue left in the flask after the distillation of the alcohol and trimethylamine consisted of the ethyl dialkylated-oxamates, with traces of ethyl monalkylated-oxamates and oxamides of primary amines. It was treated with water, which caused hydrolysis; and, on neutralising the liquid with milk of lime, calcium ethyloxamate and propyloxamate were thrown down, which on distillation with potassium hydroxide yielded ***ethylamine***, and ***propylamine***. On treating the filtrate from the calcium oxamates precipitate with an equal volume of alcohol, a precipitate was formed from which warm water extracted calcium dimethyloxamate, yielding ***dimethylamine***, on distillation with potash, while the less soluble portion consisted of calcium monomethyloxamate, yielding ***methylamine*** under similar treatment.

Ethylamine, which escaped detection on Duvillier and Buisine's first examination of the bases from vinasses, owing to the small proportion present, was subsequently detected by distilling with potassium hydroxide the mother-liquors obtained by treating the oxamides with water, and converting the bases into sulphates. On treating these with absolute alcohol, methylamine sulphate remained. On distilling the soluble portion with alkali, collecting the bases in absolute alcohol, and treating the solution with ethyl oxalate, as already described, the ethylamine was converted into a monoethyloxamate, from which the calcium salt was prepared and decomposed by alkali.

passed through a reflux condenser and a potash tower and is then collected in two wash bottles containing alcohol which must be kept well cooled. Methyl bromide passed into the cooled alcoholic solution until the liquid is no longer alkaline. The precipitated tetramethyl ammonium bromide is separated, washed with alcohol and dried; then it is treated with moist silver hydroxide and heated in a distillation apparatus from which the air has been displaced by hydrogen, and distilled in a slow current of that gas. The distillate contains methyl alcohol and trimethylamine; the former is condensed and collected in a flask and the latter, after passing through a potash tower, is collected in alcohol.

Physical Properties (see table, page 282)

When pure and concentrated, trimethylamine is stated to have a purely ammoniacal odour; but when highly diluted, the vapour has at the same time a smell of ammonia and a peculiar fishy odour suggestive of herring-brine. The latter odour is gradually developed by adding lime to a solution of the base, but requires some time to reach its maximum intensity (L. Taylor).

Trimethylamine is apparently soluble in all proportions of cold water.[1]

A mixture of equal volumes of trimethylamine and water is inflammable.

Trimethylamine might, *prima facie*, be supposed the active agent in Wollheim's process of treating sewage with herring-brine and lime (*Eng. Patent* No. 15321, 1888); but those who have investigated the lated substance which they turn aminol, produced by the action of lime on one of the amines of herring-brine. Pure methylamine employed without lime has not the same effect.

Trimethylamine is distinguished from the primary and secondary methylamines by its negative reaction with alcoholic potash and chloroform, ethyl oxalate, and nitrous acid, and by its solution in excess of hydrochloric acid being precipitated by potassium ferrocyanide.

[1] According to Guthrie, the solubility of trimethylamine in water is notably diminished by heating, the liquid becoming distinctly turbid (compare nicotine) from partial separation of the base. Thus a 10% solution of trimethylamine in water became turbid at 22°; an 8% at 24.5°, and a 4% solution at about 42°. Leo Taylor failed to confirm Guthrie's observations, which were not improbably made on impure material (see, however, under *Triethylamine*).

Trimethylamine in the form of the hydrochloride has been employed in medicine, as a specific for gout and rheumatism, but is now rarely prescribed.

Trimethylamine combines with carbon disulphide at the ordinary temperature with great evolution of heat, according to the equation $CS_2 + (CH_3)_3N = N(CH_3)_2.CS.S.CH_3$. The product, perhaps trimethyl-thiocarbamic acid, is prepared more readily by passing gaseous trimethylamine into a mixture of carbon disulphide and alcohol. It is obtained, on evaporating the solvent, in white rhombic needles, m. p. 125°, and decomposes gradually at the ordinary temperature. It is soluble in dilute alcohol and water, but nearly insoluble in absolute alcohol, ether, chloroform, or benzene. Dilute acids combine with it to form salts, but strong acids and alkalies decompose it into carbon disulphide and trimethylamine.

Trimethylamine hydrochloride, $(CH_3)_3HNCl$, is obtained by neutralising trimethylamine with hydrochloric acid. It differs from ammonium chloride is being extremely deliquescent and soluble in absolute alcohol. The fishy odour of the base liberated on treating the salt with lime or alkali hydroxide further distinguishes it from ammonium chloride. With platinum tetrachloride it unites to form the platinichloride, $(Me_3HN)_2PtCl_6$, which crystallises in orange octahedra, sparingly soluble in absolute alcohol.

When heated to 260–285°, trimethylamine hydrochloride is decomposed with formation of free trimethylamine, ammonia, and methyl chloride: $3Me_3HNCl = 2Me_3N + H_3N + 3MeCl$. This reaction has been utilised by Camille Vincent for the manufacture of methyl chloride. The vapours are passed through hydrochloric acid, which absorbs the bases, while the gaseous methyl chloride passes on. It is washed by dilute sodium hydroxide and dried by strong sulphuric acid, after which it is collected in a gas-holder, from whence it is pumped into strong wrought-iron cylinders, in which it is condensed to liquid. The vapour of liquid methyl chloride has a tension of 2.5 atmospheres at 0° and 4.8 at 20°.

Separation and Estimation of the Three Methylamines and Ammonia

A method has been described by Bertheaume (*Compt. Rend.*, 1910, **150**, 1251) which is based on the insolubility of ammonium chloride and methylamine hydrochloride in chloroform. 1 to

2 grm. of the mixed hydrochlorides, dried at 110°, are dissolved in a small quantity of water acidified with hydrochloric acid and the solution thoroughly mixed with at least 20 grm. of quartz sand. The mixture is thoroughly dried in a vacuum desiccator, extracted with pure warm chloroform, the extract evaporated to dryness and the residue weighed and dissolved in 2000 times its weight of water. A known quantity of the solution (200 to 300 c.c. at most) is cooled to 0° and a solution of iodine in potassium iodide (127 grm. iodine, 150 grm. potassium iodide in 1000 c.c. water), also cooled to 0°, is added in such proportion that at least 30 c.c. are used per 100 c.c. of the methylamine solution. The mixture is left at 0° during 1 hour, the crystals of trimethylamine periodide are filtered off in a funnel plugged with glasswool, drained, and washed with a cold mixture of the above iodine solution and water (1:3). The crystals are then dissolved in normal sodium sulphite solution, the solution distilled with excess of sodium carbonate in a Schloesing apparatus, and the trimethylamine estimated volumetrically in the distillate. The dimethylamine is similarly estimated in the mother liquor from the trimethylamine periodide. The residue insoluble in chloroform is dried to remove chloroform and then extracted with hot water. In the solution the methylamine and ammonia are separated by François' method (*Compt. Rend.*, 1907, **144**, 857) as follows:

0.5 grm. of the dry hydrochloride is placed in a 250 c.c. flask and 7 c.c. of a 30% solution of sodium hydroxide, 10 c.c. of a 20% solution of sodium carbonate and 5 grm. of yellow mercuric oxide added. The mixture is diluted to the mark with water and agitated for 1 hour. The freedom of the supernatant liquid from ammonia should then be ascertained by adding a few c.c. to a Nessler solution made by dissolving 22.7 grm. of mercuric iodide, 33 grm. of potassium iodide and 35 grm. of sodium hydroxide in 1 litre of water. The Nessler solution, when heated to boiling, gives a reddish-brown precipitate if a liquid containing as little as 0.002% of ammonium chloride is added, but gives no precipitate with methylamine. The methylamine in the supernatant liquid free from ammonia is then estimated by Schloesing's method, with the use of litmus as indicator and barium hydroxide as standard alkali. The ammonia remains in combination with mercuric oxide, and may be obtained by washing the latter with water containing sodium hydroxide and carbonate, placing it in a Schloesing's apparatus and adding 50 grm. of potassium

iodide. The liberated ammonia is then estimated in the usual manner.

In presence of large quantities of ammonia and only relatively small proportions of the amines, the above-described process of separating the methylamines needs modifying, Jarry's method of eliminating ammonia being employed (Bertheaume, *Comp. Rend.*, 1910, **151**, 146). The liquid is placed in the first of a series of 4 Durand wash bottles, (600–800 c.c. capacity) with about 6 times the quantity of hydrochloric acid necessary to neutralise the estimated quantity of amines present. A similar quantity of hydrochloric acid, diluted to 50–100 c.c. with water, is placed in each of the other 3 wash bottles. 2 large wash bottles (each of 1000 c.c. capacity) containing 1:1 hydrochloric acid sufficient to neutralise the whole of the ammonia, complete the series. A rapid current of air is aspirated through the vessels until the contents of the first 4 are neutral: these 4 now contain the whole of the mono- and dimethylamines together with a little ammonium chloride. The united liquids are evaporated to a few c.c., mixed with quartz sand and treated by the method already given for separating methylamine and dimethylamine. The other 2 wash bottles contain the trimethylamine and the whole of the ammonia, save the small portion remaining with the other amines. The liquid is evaporated to 500 c.c., cooled to 0°, excess of ammonium chloride separated and the trimethylamine estimated as periodide.

Trimethylamine in the presence of ammonia can be estimated by taking advantage of the fact that it does not react with formaldehyde (Budai, *Zeitsch. Physiol. Chem.*, 1913, **86**, 107). The aqueous solution of the hydrochlorides of the two bases is treated with excess of formalin, previously made neutral to phenolphthalein and is then titrated with N/10 sodium hydroxide in presence of phenolphthalein; the nunber of c.c. used (x) corresponds with the *ammonia*.

$$4NH_4Cl+6CH_2O+4NaOH=(CH_2)_6N_4+10H_2O+4NaCl$$

The solution is now diluted with a large quantity of water, made strongly acid with sulphuric acid and concentrated to one-third of its volume over a naked flame. The hexamethylenetetramine produced by the action of the formaldehyde on the ammonium salt is in this way hydrolysed into ammonia and formaldehyde. The solution is now made alkaline and distilled, the vapours being collected in excess of N/10 acid, the excess of acid subsequently remaining being titrated. From this titration the number of c.c. (y) of the N/10

alkali corresponding with the ammonia and trimethylamine is ascertained, and hence $y - x$ gives the value corresponding with the trimethylamine.

Ethylamines

The ethylamines are obtainable in the manner already described (page 271). A convenient source of the primary amine, $C_2H_5.NH_2$, is the crude ethyl chloride obtained as a by-product in the manufacture of chloral (A. W. Hofmann, *Ber.*, 3, 109, 776). When ethyl chloride is heated to 90° under pressure with an equivalent proportion of strong aqueous ammonia, a layer of triethylamine containing ammonia is formed, while the aqueous liquid contains the hydrochlorides of ethylamine and diethylamine. When a similar mixture of aqueous ammonia and ethyl chloride is heated under pressure to 150°, H_4NCl, EtH_3NCl, and Et_4NCl are the chief products, only traces of Et_2H_2NCl and Et_3HNCl being formed.

The ethylamines can be separated by methods already described. They present the closest analogy to the corresponding methyl bases. Various differences between the 3 amines are described on pages 272 and 277. The following table shows other of their characteristic properties.

	Ethylamine	Diethylamine	Triethylamine
Formula	$(C_2H_5)H_2N$	$(C_2H_5)_2HN$	$(C_2H_5)_3N$
Boiling-point	19°	56°	90°
Specific gravity	0.6964 (8°)	0.7262 (0°)	0.7426 (4°)
	0.6892 (15°)	0.7107 (15°)	0.7331 (15°)
Reaction with zinc sulphate.	Precipitate soluble in excess.	Precipitate insoluble in excess.	Precipitate insoluble in excess.
Product when boiled with nitrous acid (or a salt of the bases with sodium nitrite solution).	Alcohol and nitrogen.	Diethylnitrosamine; a neutral oily liquid boiling at 177°, and distilling with steam.	Unchanged.
Hydrochloride	Deliquescent laminæ and prisms.	Non-deliquescent plates.	Non-deliquescent laminæ.
Platinichloride	Hexagonal rhombohedra; moderately soluble in water.	Monoclinic; moderately soluble.	Monoclinic; very soluble.
Acid ferrocyanide	Soluble	Soluble	Very sparingly soluble.

Triethylamine mixes with water in all proportions below 18°, but, on raising the temperature the solution becomes turbid and separates into two layers. For the mutual solubility of triethylamine and water see Rothmund (*Zeitsch. Phys. Chem.*, 1898, **26**, 433).

Volatile alkylamines often occur in foodstuffs which are in a state of incipient putrefaction; hence their recognition is a matter of importance. They are, however, difficult to distinguish from ammonia when present only in small quantities. Woodward and Alsberg (*J. Biol. Chem.*, 1921, **46**, 1) give a useful method which is available for this purpose; it is based on the fact that, while ammonia forms hexamethylenetetramine with formaldehyde, the alkylamines form alcohol and acids, which latter may be titrated. The distillate from a quantity of the foodstuff is collected in a slight excess of acid, and the acid solution is evaporated to a low bulk, made alkaline and then distilled into about 1 c.c. of formalin contained in a test tube. One c.c. of a solution containing 12 per cent. potassium bromide and 18 per cent. of mercuric bromide is added, and the mixture gently warmed. In the presence of 0.5 mg. or more of amino-nitrogen there is produced a white precipitate of mercurous bromide insoluble in excess of formalin. The reaction may be made approximately quantitative by using about 10 c.c. of solution containing between 0.01 N and 0.005 N weight of the amine, the precipitate then weighs 20 times the amino-nitrogen. When tertiary amines are present with the primary and secondary bases the acid solution of the distillate is evaporated, filtered and precipitated by the gradual addition of Mayer's[1] reagent, each c.c. of which precipitates 59 mg. of trimethylamine.

Tetralkylammonium Bases

Tetralkylammonium *iodides* result from the action of alkyl iodides on tertiary amines; action generally takes place at the ordinary temperature, with evolution of heat. Trimethylamine combines in the same way with methyl chloride to form NMe_4Cl, but it does not combine with ethyl chloride at the ordinary temperature even under a pressure of 50 atm. The tetralkylammonium *chlorides* as a rule are best obtained by digesting the corresponding iodides with silver chloride: $NMe_4I + AgCl = AgI + NMe_4Cl$. In a similar way the *sulphates* may be obtained with the aid of silver sulphate. All

[1] The Mayer's reagent should contain 45 grm. of mercuric iodide and 33 grm. of potassium iodide per 100 c.c.

these salts are crystalline compounds which generally dissociate on heating, giving the tertiary amines: $NMe_4I \rightleftarrows NMe_3 + MeI$. In the decomposition of mixed ammonium haloids containing methyl groups, the methyl radical generally separates from the nitrogen atom, *e. g.*, $NMeEt_3Cl = NEt_3 + MeCl$.

The tetralkylammonium iodides combine with iodine to form intensely coloured tri-iodides, penta-iodides, hepta- and ennea-iodides, *e. g.*, NMe_4I, I_2; NMe_4I, I_4; etc.

The tetra-alkylammonium iodides cannot be decomposed by aqueous potassium hydroxide, even on heating, but react with freshly precipitated silver oxide to form silver iodide and the tetra-alkyl ammonium hydroxides. These hydroxides are non-volatile, syrupy or solid deliquescent substances, of highly caustic, alkaline character, presenting, as a class, a strong analogy with potassium hydroxide. Many of them have marked poisonous characters.

It is possible to liberate the tetralkylammonium bases from their halogen salts by potassium hydroxide if a solvent is used in which the potassium haloid is sparingly soluble. Thus, for example, in methyl or ethyl alcohol, the action $NMe_4Cl + KOH = NMe_4.OH + KCl$ takes place with precipitation of potassium chloride. (Walker and Johnson, *Trans.*, 1905, **87**, 955.) On filtering, adding a little water, and concentrating *in vacuo*, crystalline hydrates are obtained. The *hydrate*, $NMe_4.OH, 5H_2O$, has m. p. 62–63°; 100 parts water dissolve 151 parts at 0° and 220 parts at 15°. $NMe_4OH, 3H_2O$ has m. p. 59–60° and when warmed in a vacuum at 35° gives NMe_4OH, H_2O, which decomposes when heated at 130–135°, forming trimethylamine.

It is noteworthy that the mixed tetralkylammonium bases containing methyl, *unlike the haloids*, generally retain methyl in combination with nitrogen when decomposed by heat, while ethyl groups separate in the form of ethylene, *e. g.*,

$$NMe(C_2H_5)_2(C_5H_{11}).OH = NMe(C_2H_5)(C_5H_{11}) + C_2H_4 + H_2O.$$

Tetrethylammonium iodide $(C_2H_5)_4NI$, is prepared by exposing a mixture of equivalent proportions of triethylamine and ethyl iodide to a temperature of 100° for a few minutes in a flask fitted with a reflux apparatus, or preferably in a sealed tube. Violent action ensues, and, on cooling, the product sets to a dark mass of crystals. On dissolving in water, and allowing the solution to evaporate spontaneously, the iodide is obtained in extremely bitter

crystals of considerable size, which, when pure, are colourless, but are apt to be mixed with reddish crystals of the tri-iodide, $(C_2H_5)_4NI, I_2$.

Tetrethylammonium iodide is not volatile at 100°, but when rapidly heated in a retort to a higher temperature melts and is decomposed into ethyl iodide and trimethylamine, which form separate layers in the receiver but re-unite to produce the original compound.

Tetrethylammonium iodide is not apparently decomposed by treatment with potassium or sodium hydroxide, but is much less soluble in caustic alkaline solutions than in water. Hence, on adding excess of potassium hydroxide to its concentrated aqueous solution, a solid crystalline mass is produced. This behaviour sharply distinguishes the iodide of tetrethylammonium (and of other compound ammoniums) from the compounds Et_3HNI, Et_2NHI, and EtH_3NI, which are at once decomposed by alkali hydroxide with liberation of the corresponding amines. The aqueous solution of tetrethylammonium iodide reacts with silver nitrate or sulphate to form a precipitate of silver iodide and a solution of the tetrethylammonium nitrate or sulphate.

Tetrethylammonium hydroxide, $(C_2H_5)_4N.OH$, is obtained in solution by adding freshly-precipitated oxide of silver to a dilute and warm solution of tetrethylammonium iodide, until the brown colour of the silver oxide ceases to change into the lemon-yellow of the iodide. The solution is then filtered, and may be evaporated to a considerable extent at a gentle heat, but further concentration must be conducted *in vacuo*, at the ordinary temperature, over sulphuric acid and lime. Long, hair-like, deliquescent needles of the base are deposited, but these subsequently disappear, and the liquid ultimately dries up to a semi-solid mass.

Tetrethylammonium hydroxide presents the closest analogy to potassium hydroxide. It is highly deliquescent, absorbs carbon dioxide from the air, and the aqueous solution has a strong alkaline reaction. It has an alkaline, caustic, and extremely bitter taste, and in a concentrated state burns the tongue and acts on the skin like potassium hydroxide. With metallic solutions it behaves like the alkali hydroxides except that aluminum hydroxide is soluble with difficulty in excess of the base and chromium hydroxide is insoluble.

A moderately concentrated solution of tetrethylammonium hydroxide may be boiled without decomposition; but in a concen-

trated state, even at 100°, the liquid froths strongly, and the base is resolved gradually but completely into triethylamine, ethylene, and water: $(C_2H_5)_4N.OH = (C_2H_5)_3N + C_2H_4 + H.OH$. This action affords a convenient means of obtaining triethylamine unmixed with primary and secondary amines.

When a solution of tetrethylammonium hydroxide is boiled with a slight excess of ethyl iodide for 24 hours, under a reflux condenser, the solution becomes perfectly neutral, the following action occurring: $(C_2H_5)_4N.OH + C_2H_5I = (C_2H_5)_4NI + C_2H_5.OH$.

Tetrethylammonium hydroxide also hydrolyses ethyl oxalate, and saponifies fats as readily as potassium hydroxide.

On adding potassium hydroxide and potassium iodide to a strong solution of tetrethylammonium hydroxide, a white crystalline mass of tetrethylammonium iodide is produced.

The *salts* of tetrethylammonium are mostly crystallisable and readily soluble.

Tetrethylammonium chloride, $(C_2H_5)_4NCl$, obtained by neutralising the hydroxide with hydrochloric acid, is crystalline and highly deliquescent. If forms double salts with auric, mercuric, and platinic chlorides. *Tetrethylammonium platinichloride*, $(Et_4N)_2PtCl_6$, is thrown down immediately as an orange-yellow precipitate, consisting of microscopic octahedra, on adding platinic chloride to a solution of tetrethylammonium chloride. It is slightly soluble in water, and less soluble in alcohol and ether.

For the resolution of asymmetric tetralkylammonium compounds into optically active components by means of *d*-camphorsulphonic acid. (See Pope and Peachey, *J. Chem. Soc.*, 1899, **75**, 1127, and Pope and Harvey, *ibid.*, 1901, **79**, 828.)

Diamines

As already indicated, these are substances containing two amino groups replacing two hydrogen atoms. They are strongly basic substances, having properties analagous with those of the corresponding primary compounds but the m. p. and b. p. are much higher. The substituted diamines are generally stable in dry air, but in solution they are readily oxidised.

Di-amino-acids are important as being a product of the decomposition of protein in foodstuffs, and some of the so-called

ptomaines belong to this class. Putrescine and cadaverine are respectively tetra- and penta-methylene diamine.

The methods of preparation are generally similar to those used for the monoamines and, in the case of aromatic diamines, the reduction of the appropriate di-nitro compound. The more important diamines are discussed in Vol. VII sections on Animal bases and Ptomaines.

HYDRAZINES

The parent substance of this group is diamide $H_2N.NH_2$, from which five classes of hydrazines can be formed and, indeed, are known: 1. $RHN.NH_2$, 2. $RR.N.NH_2$, 3. $RHN.NHR$, 4. $RRN.$-NHR, and 5. $RRN.NRR$; the radicals may be either aliphatic or aromatic; but usually the latter are more important. The five classes mentioned are, like the amines, 1. primary 2. secondary 3. asymetrical secondary 4. tertiary and 5. quaternary. The general method of production is the preparation of the nitroso derivative of the corresponding amine and its reduction with zinc dust stannous chloride or alkali sulphite.

The primary hydrazines are strongly basic, forming mono-salts which are usually stable; they are strong reducing agents and readily reduce Fehling solution, even when cold. The secondary hydrazines are also mono-bases, but their salts are relatively unstable. The tertiary and quaternary compounds are not so important commercially.

Hydrazine.—Diamide: N_2H_4 or $H_2N.NH_2$ is obtained by the decomposition of triazo-acetic acid by heating it with water or mineral acids, when the following action occurs:

$$\underset{\text{Triazo-acetic acid.}}{C_3H_3N_6(COOH)_3} + \underset{\text{Water.}}{6H_2O} = \underset{\text{Hydrazine}}{3N_2H_4} + \underset{\text{Oxalic acid.}}{3C_2H_2O_4}.$$

The oxalic acid is more or less split up, according to the temperature and the strength of the acid employed, into carbonic and formic acids, so that when only water is used the hydrazine separates as a formate; but if a mineral acid be present it forms the corresponding salt.

Hydrazine hydrate (*infra*) is best prepared (Curtius and Schulz) by distilling a mixture of 11 parts of hydrazine sulphate with 4 of potassium hydroxide and 1 of water in a silver retort provided with a silver condenser. When the last drop has passed over, the dis-

tillate is fractionated. After four fractionations the last portions boil constantly at 119°. Curtius and Jay (*J. prakt. Chem.*, **39**, [ii], 27) prepare hydrazine hydrate by heating the hydrochloride of the base with calcium oxide in a silver retort, and passing the vapours through a heated silver tube containing anhydrous lime.

Free hydrazine, $NH_2.NH_2$, is obtained by decomposing the hydrate with barium oxide or from its hydrochloride by the action of sodium in absolute ethereal or methyl alcoholic solution (Lobry de Bruyn, *Rec. Trav. Chim.*, 1894, **13**, 433; 1896, **15**, 174). It has b. p. 113.5°/761.5 mm.; 56°/71 mm.; sp. gr. 1.014 at 15°.

A rapid means of preparing hydrazine sulphate for laboratory purposes is given by Raschig, based on the action of ammonia on monochloramine, NH_2Cl, prepared by the interaction of ammonia and sodium hypochlorite; for details see Joyner, *J. Chem. Soc.*, 1923, **123**, 1114.

Hydrazine has an extraordinary affinity for water, readily forming the hydrate N_2H_4,H_2O. This is a liquid which fumes in the air, b. p. 119°.

The solution of hydrazine turns reddened litmus-paper a deep blue, and gives white fumes with acid vapours. In a concentrated state it has a very peculiar odour, only slightly resembling that of ammonia. It powerfully affects the nose and throat, has an alkaline taste, and leaves a burning sensation on the tongue. When boiling, the solution attacks glass, and quickly destroys corks and india-rubber. Hydrazine, like hydroxylamine, is a strong poison of universal character.

Hydrazine reduces Fehling's solution and ammoniacal silver nitrate in the cold. With copper sulphate it yields a red precipitate, with mercuric chloride a white precipitate, and it precipitates alumina from a solution of alum. With aromatic aldehydes and ketones it yields sparingly soluble crystalline compounds.

Salts of Hydrazine

Hydrazine combines with 1 or 2 molecules of monobasic acids to form very stable salts, which are usually crystalline and isomorphous with the corresponding ammonium salts. The salts Hz,2HR crystallise in the cubic system and are readily soluble in water, but nearly insoluble in alcohol. The mono-acid salts, HzHR, are easily

soluble in water and warm alcohol, from which they crystallise well. The salts of both classes are insoluble in ether, benzene, etc. In acid solution, the salts of hydrazine possess remarkably strong reducing properties, and are powerfully toxic toward the lower organisms. Peptone solutions containing 0.1% of hydrazine sulphate are unable to support bacterial life.

Hydrazine dihydrochloride, $N_2H_4,2HCl$, crystallises from hot water in large glassy octahedra which are freely soluble in water, but less so in alcohol. On treatment with platinum tetrachloride it does not yield a platinichloride, but is decomposed with evolution of nitrogen. It melts at 198°, with evolution of hydrochloric acid, to a clear glass consisting of the *monohydrochloride*, N_2H_4HCl, and this, on further heating to 240°, is decomposed into ammonium chloride, nitrogen, and hydrogen.

Hydrazine sulphate, N_2H_4,H_2SO_4, is somewhat sparingly soluble in water.

Hydrazine nitrate, $N_2H_4HNO_3$, melts at 70°, and may be kept at 100° for a long time without decomposition. Heated to 200° *in vacuo* it decomposes in accordance with the equation $4N_2H_4HNO_3 \rightarrow 5N_2 + 2NO + 10H_2O$; heated in a closed vessel it explodes, but under ordinary pressure merely burns rapidly. Contact with oxidisive agents, such as permanganates or peroxides, causes ignition. A solution of the nitrate does not act on zinc, cadmium or magnesium; these metals are however rapidly dissolved by ammonium nitrate solution. Zinc, cadmium or copper added to the fused salt at 70° cause flaming decomposition. Fragments of ordinary commercial cube cobalt or nickel added to the fused salt cause a violent explosion, but this behaviour is not shown by the same metals that have been melted and worked into wire, nor by these metals when prepared by reducing the oxides in hydrogen. In the latter case the metal is only slightly oxidised when added to the fused nitrate, which burns away rapidly. (C. F. Hodgkinson, *J. Soc. Chem. Ind.*, 1913, **32**, 519.)

Salts of hydrazine in solution are decomposed by sodium nitrite, with evolution of gas, attended by much frothing. The reaction is analogous to the decomposition of ammonium salts by a nitrite, with the difference that whereas in the latter case (*a*) nitrogen is formed, in the case of hydrazine (*b*) azoimide, HN_3, is found among the products of the action:

$$NH_3,HCl + NaNO_2 = NaCl + 2H_2O + N_2. \qquad (a)$$
$$N_2H_4,HCl + NaNO_2 = NaCl + 2H_2O + HN_3. \qquad (b)$$

Detection of Hydrazine

1. Benzaldehyde gives with its solutions, alkaline or acid, dilute or concentrated, yellow flocks of *benzalazine*, CHPh:N.N:CHPh, m. p. 93°. 2. In solutions more dilute than 1:2,000, copper sulphate gives a sparingly soluble blue double salt, $CuSO_4,(N_2H_5)_2SO_4$ (Curtius and Schrader). 3. Hydrazine reduces gold chloride in acid solution, a fact which distinguishes it from hydroxylamine.

Estimation

1. By measuring the iodine absorbed by a known volume of its solution (Curtius and Schulz). This is obviously practicable only in the absence of other substances which absorb iodine. According to Stolle (*J. pr. Chem.*, 1902, **66**, 332) by hydrazine or its salts can readily be titrated with iodine solution in presence of sodium hydrogen carbonate: the action is $N_2H_4 + 2I_2 = N_2 + 4HI$. The sodium hydrogen carbonate is added and the solution immediately titrated with standard iodine in presence of starch as indicator. As the action finishes slowly, the final coloration should persist at least 2–4 minutes. Rupp (*J. pr. Chem.*, 1903, **67**, 140) advises dissolving the hydrazine sulphate in aqueous potassium hydrogen carbonate, leaving for 15 minutes with excess of N/10 iodine and then estimating the excess of iodine with sodium thiosulphate. The use of potassium tartrate or sodium acetate in place of potassium hydrogen carbonate is said to give better results.

2. By measuring the nitrogen evolved when shaken with Fehling's solution.

3. By measuring the potassium permanganate required for its oxidation in sulphuric acid solution (6–12%). (Petersen, *Zeitsch. anorg. Chem.*, **5**, 1.)

4. **Iodate Method.**—This method which is described by Kolthoff (*J. Amer. Chem. Soc.*, 1924, **46**, 2009) depends upon the following reaction:

$$N_2H_4.H_2SO_4 + KIO_3 + 2HCl = N_2 + ICl + KCl + H_2SO_4 + 3H_2O.$$

Such quantity of solution as will contain 0.05 to 0.10 grm. of hydrazine sulphate is added to an equal volume of hydrochloric acid and

6 c.c. of chloroform. Potassium iodate solution (3.567 grm. per litre) is run in slowly, with frequent shaking, until the chloroform is just decolorised. Each c.c. of iodate solution is equivalent to 0.000534 grm. N_2H_4. The metallic hydrozine salts such as Zn, Cd, Ni, or $CaSO_4(N_2H_4)_2.H_2SO_4$ can be estimated in the same manner.

5. According to Stollé (*loc. cit*) hydrazine sulphate can be titrated with potassium hydroxide, with methyl orange as indicator; the action which occurs is as follows:

$$2N_2H_4,H_2SO_4 + 2KOH = (N_2H_4)_2,H_2SO_4 + K_2SO_4 + 2H_2O.$$

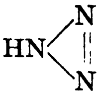
6. Ebler's gasometric method (*Zeit. anorg. Chem.*, 1905, **47**, 377) is based on the reduction of mercuric salts, for example mercuric chloride, according to the equation: $N_2H_4 + 2HgCl_2 = 4HCl + 2Hg + N_2$. The mercuric salt is dissolved in 10 c.c. of dilute hydrochloric acid, 5 grm. of sodium acetate dissolved in 10 c.c. of water are added and the whole introduced into a flask (500–700 c.c.) fitted with a ground stopper carrying 1. a tap funnel, 2. a tube passing to the bottom of the flask through which CO_2 can be introduced, and 3. a reflux condenser the inner tube of which can be connected with a Schiff's nitrometer. Carbon dioxide is passed through the flask until all air is expelled, the liquid being maintained just below 100°. The solution of the hydrazine salt is then added very gradually, and CO_2 is passed continuously until the volume of gas in the nitrometer ceases to increase. The volume of nitrogen may be measured direct in the nitrometer if graduated in the usual way, or the gas may be transferred to a Hempel's gas burette.

7. Hofmann and Küspert (*Ber.*, 1898, **31**, 64) measure the nitrogen evolved from the hydrazine salt when oxidised by vanadic acid, thus, $N_2H_4 + O_2 = N_2 + 2H_2O$; or the amount of vanadic acid used in the oxidation may be estimated by means of permanganate.

8. Sommer (*Zeit. anorg. Chem.*, 1913, **83**, 119) shows that according to Sommer hydrazine may be estimated in presence of nitrous acid by titrating with iodine after adding bicarbonate, and the amount of nitrous acid may then be found by adding potassium iodide, displacing oxygen by carbon dioxide, adding dilute sulphuric acid, and titrating with thiosulphate.

9. Hydrazine is oxidised by a bromate solution in hydrochloric acid completely and instantaneously to nitrogen, it may therefore, be estimated by adding potassium bromide to the solution, then

hydrochloric acid and titrating with 0.1 N potassium bromate solution at 60°; the best indicator is indigo, added towards the end of the titration, which is continued until it just turns yellow. This method is available for the estimation of hydrazine in the presence of hydroxylamine (see Kartenachar and Wagner. *Zeit. anorg. Chem.*, 1921, **120**, 261).

Azoimide. Iminazoic Acid. HN_3 = 

Azoimide is obtained in the form of its sodium derivative, with a yield of 50%, by the action of nitrous oxide on sodamide: $N_2O + NH_2Na = NaN_3 + H_2O$ (Wislicenus). It can also be obtained by passing nitrous fumes into a solution of hydrazine sulphate at 0°: $NH_2.NH_2 + HNO_2 = N_3H + 2H_2O$ (Angeli). Curtius, who discovered it, prepared it (*Ber.*, 1890, **23**, 3023) by decomposing nitrosohippurylhydrazine, $NHBz.CH_2.CO.N(NO).NH_2$, with dilute sodium hydroxide, which splits it up into hippuric acid and the sodium salt of azoimide:

$$NHBz.CH_2.CO.N(NO).NH_2 + 2NaHO = NHBz.CH_2.COONa + 2H_2O + NaN_3.$$

On distilling the compound NaN_3 with dilute sulphuric acid, azoimide volatilises with the steam, which when passed into a neutral solution of silver nitrate gives a precipitate of the silver salt. This is washed and decomposed by dilute sulphuric acid, this solution being used instead of silver nitrate to absorb the vapours of azoimide. By repeating this process, a solution containing 27% of the new acid is obtainable. It can be obtained by several other methods.

In the anhydrous state, azoimide is a colourless gas of a peculiarly nauseous odour, and condensible, on cooling, to an extremely explosive liquid which boils at 37°. It is very soluble in water, and on distillation of the liquid a concentrated acid passes over, the distillate gradually becoming weaker until an acid of constant composition and b. p. distils. The solution reddens litmus, and gives white fumes with ammonia, of the salt $NH_3.HN_3$ or N_4H_4, which sublimes completely at 100°, but does not crystallise in the cubic system like ammonium chloride. Iron, zinc, copper, aluminium and magnesium dissolve readily in dilute iminazoic acid (7%) with evolution of

hydrogen, and gold is dissolved with formation of a red salt. The *silver* (AgN_3) and *mercurous salts* of iminazoic acid are insoluble, the former closely resembling silver chloride, but not blackening in the light. Both the silver and the mercurous salts are extraordinarily explosive, 0.001 grm. of the former indenting an iron plate on which it is heated to 250°. *Barium azoimide*, BaN_6, separates from concentrated solutions in short shining anhydrous crystals, which explode with a green flash when heated or exposed to a strong green light. The solution of *cupric azoimide* deposits cuprous oxide on boiling. The free acid is liberated from any of the iminoazoates by treatment with dilute sulphuric acid. By concentrated sulphuric acid, the azoimide is itself decomposed. *Esters* of iminazoic acid have been prepared, phenyl iminazoate, PhN_3, being identical with the diazobenzolimide previously described by Griess.

SUBSTITUTED HYDRAZINES

Hydrazine is the parent of a large and important class of bases generally called hydrazines, one member of which, phenylhydrazine, $(C_6H_5)NH.NH_2$, has proved, in the hands of E. Fischer and others, a reagent of the highest importance. By replacing a second atom of hydrogen by (*e. g.*) phenyl, secondary hydrazines may be obtained either symmetrical like hydrazobenzene, $(C_6H_5)HN.NH(C_6H_5)$, or unsymmetrical like diphenylhydrazine, $(C_6H_5)_2N.NH_2$. The latter class resemble the tertiary amines in their power of reacting with the haloid salts of the alkyl radicals (*e. g.*, ethyl iodide) to form hydrazonium compounds, $R_2N.NH_2 + AlkI = IAlkR_2N.NH_2$.

The hydrazines containing fatty alkyl-radicals are liquids boiling without decomposition; those of the aromatic series are readily fusible solids or oily liquids, and are partially decomposed on distillation. Hydrazine itself and some of the fatty derivatives are di-acid bases; but the hydrazines of the benzene series have all monobasic functions.

The hydrazines closely resemble the amines, but are distinguished from them by their capacity of reducing Fehling's copper solution, in many instances at the ordinary temperature. The product of the oxidation of the hydrazine is the corresponding amine. Thus, diethylhydrazine, $(C_2H_5)_2N.NH_2$, is oxidised to diethylamine. $(C_2H_5)_2HN$.

The general and special characters of the hydrazines are sufficiently exemplified by two typical species, ethylhydrazine and phenylhydrazine.

Ethylhydrazine. $(C_2H_5)HN.NH_2$

On treating diethyl-carbamide with nitrous acid, a nitroso-compound is formed, which, on reduction with zinc-dust and acetic acid. is converted into diethyl-semicarbazide.

$$CO\langle\begin{array}{l}HN(C_2H_5)\\NH(C_2H_5)\end{array} \qquad CO\langle\begin{array}{l}NH(C_2H_5)\\N(NO)(C_2H_5)\end{array} \qquad CO\langle\begin{array}{l}NH(C_2H_5)\\N(NH_2)(C_2H_5)\end{array}$$

Diethyl-carbamide. Nitroso-compound. Diethyl-semicarbazide.

This carbazide decomposes, on heating with strong hydrochloric acid, into ethylhydrazine, ethylamine, and carbon dioxide:

$$NH(C_2H_5).CO.N(NH_2)C_2H_5 + H_2O = HN.(NH_2)(C_2H_5) + C_2H_5NH_2 + CO_2.$$

Primary hydrazines can also be obtained by heating the potassium salts of alkylsulphuric acids with hydrazine hydrate (Stollé, 1902) and by the reduction of nitroamines.

Ethylhydrazine hydrochloride is less soluble than the corresponding salt of ethylamine, and may be separated from it by crystallisation.

Ethylhydrazine is a colourless, mobile liquid of ethereal and faintly ammoniacal odour. It boils at 99.5° under 709 mm., and distils undecomposed. It is very hygroscopic, forming white fumes with moist air, dissolves in water and alcohol with evolution of heat, and corrodes cork and caoutchouc.

Ethylhydrazine gives Hofmann's isonitrile reaction for primary amines with chloroform and alcoholic potassium hydroxide. Bromine decomposes it with evolution of nitrogen, and it is also decomposed by nitrogen trioxide.

Ethylhydrazine is a very powerful reducing agent. It reduces Fehling's copper solution at the ordinary temperature and liberates silver from its oxide. It yields a black precipitate with Nessler's solution.

Ethylhydrazine reacts with aldehydes, with evolution of heat, to form ethylhydrazones, $R.CH:N_2H(C_2H_5)$.

Potassium pyrosulphate, $K_2S_2O_7$, acts on ethylhydrazine to form potassium ethylhydrazine-sulphonate, $(C_2H_5)HN.NH(SO_3K)$, which, on treatment with mercuric oxide, gives potassium diazo-ethane-sulphonate, $C_2H_5.N:N.(SO_3K)$, a substance which explodes violently when warmed, and otherwise resembles the diazo-benzene-sulphonates.

Asymm. diethylhydrazine, $(C_2H_5)_2N.NH_2$, is obtained by the reduction of the nitroso-derivative of diethylamine: $(C_2H_5)_2N.NO + 2H_2 = (C_2H_5)_2N.NH_2 + H_2O$. It boils at 96–99°, and closely resembles ethylhydrazine, but does not reduce Fehling's solution unless the liquid is heated. It unites with ethyl iodide to form the compound $(C_2H_5)_3N_2H_2I$, which on treatment with oxide of silver yields a strongly alkaline solution of triethylazonium hydroxide, $(C_2H_5)_3N_2$-H_2OH, a powerful base analogous to tetrethylammonium hydroxide (page 294); when heated with water, this decomposes into ethylene, diethylhydrazine, and water. Mercuric oxide, even in the cold, converts asymm. diethylhydrazine into tetraethyltetrazone, $(C_2H_5)_2$-$N.N:N.N(C_2H_5)_2$, a colourless, strongly basic oil, volatile with steam, which yields a metallic mirror with ammoniacal silver nitrate.

Sym. diethylhydrazine, NHEt.MHEt, boils at 84–86°, and is converted by oxidation with mercury oxide into mercury ethyl, $HgEt_2$, nitrogen being evolved; nitrous acid transforms it into ethyl nitrite, and concentrated hydrochloric acid splits it up into ethyl chloride and ammonium chloride.

Methylhydrazine, $NHMe.NH_2$, has b. p. 87°; asymm. *dimethylhydrazine* boils at 63° and has sp. gr. 0.801/11°; sym. *dimethylhydrazine* has b. p. 50–60°.

Phenylhydrazine. $C_6H_8N_2 = (C_6H_5)HN.NH_2$

Phenylhydrazine is prepared by the action of reducing agents on diazobenzene salts, $C_6H_5N:NX$. Thus benzenediazonium chloride may be reduced by the calculated amount of stannous chloride and hydrochloric acid; or by adding to the solution of benzene diazonium chloride its equivalent of sodium sulphite and then warming it on the water bath with zinc dust and acetic acid until colourless, then filtering off the unchanged zinc, the product being subsequently decomposed by boiling with hydrochloric acid:

$$C_6H_5.HN.NH.SO_3K+HCl+H_2O = KHSO_4+C_6H_5.HN.NH_2,HCl.^{[1]}$$

[1] Phenylhydrazine is best obtained, as described by V. Meyer, by dissolving 1000 parts of aniline in 2000 parts of concentrated hydrochloric acid, cooling the solution by means of ice, and then slowly adding an ice-cold solution of 75 parts of sodium nitrite in 400 c.c. of water. To the cold solution of benzenediazonium chloride, $C_6H_5.N:N.Cl$, so obtained a solution of 450 parts of stannous chloride in an equal weight of hydrochloric acid is added. The mixture soon sets to a white crystalline pulp of phenylhydrazine hydrochloride $C_6H_5N_2H_3,HCl$, which is filtered or strained off, and washed with a mixture of alcohol and ether. The free base is obtained by dissolving the hydrochloride in water, adding sodium hydroxide, and agitating with ether, which is separated and evaporated. The product is purified by distillation.

Phenylhydrazine when freshly distilled is a colourless oil, which afterwards sets as a mass of monoclinic crystals of m. p. 19.6° (E. Fischer). The commercial article usually appears as a pale brown oil or crystals of m. p. 20–22°. It boils, with slight change and evolution of ammonia, at 241–242°. It distils unchanged at 120° under 12 mm. pressure, and volatilises in a current of steam, but not very readily. Phenylhydrazine dissolves sparingly in cold water, more readily in hot, and very readily in alcohol, ether, chloroform, and benzene.

Phenylhydrazine is readily oxidisable, and becomes red and ultimately dark brown on exposure to air, from absorption of oxygen.

Phenylhydrazine has well-marked antiseptic properties, and a 0.1 % solution of the hydrochloride has been recommended as a substitute for one of mercuric chloride of equal strength (*Pharm. J.* [*v*], **19**, 608).

Under certain undetermined conditions, contact of phenylhydrazine with the skin produces troublesome sores.

Phenylhydrazine has well-marked basic properties, and forms well-crystallised salts. The *hydrochloride* crystallises from hot water in small, thin, lustrous plates, and is almost completely precipitated from its aqueous solution by concentrated hydrochloric acid, a reaction by which phenylhydrazine may be readily separated from aniline and several other bases. If carefullv heated, it can be sublimed without decomposition.

Solutions of the hydrochloride and other salts of phenylhydrazine act as powerful reducing agents. They reduce the salts of silver, mercury, gold, and platinum in the cold. Freshly-precipitated mercuric oxide is reduced, a salt of diazobenzene being reproduced. Fehling's solution is reduced in the cold, with evolution of nitrogen and precipitation of cuprous oxide, aniline and benzene being simultaneously formed.

Phenylhydrazines as a class are converted by aqueous copper sulphate into the corresponding aromatic hydrocarbon. This reaction affords a ready means of replacing an amino-group in an aromatic nucleus by hydrogen, the aromatic base being first converted into the corresponding hydrazine, which in the form of its hydrochloride or sulphate is added to a boiling solution of copper sulphate.

If phenylhydrazine hydrochloride is treated with a cold solution of potassium nitrite, a nitroso-compound, $C_6H_5(NO)N.NH_2$, separates

in yellow flocks, which, on treatment with phenol and strong sulphuric acid, yield a brown solution, changing to green and blue. (Liebermann's test for nitroso-derivatives.)

Phenylhydrazine combines directly with carbon dioxide, carbon disulphide, and cyanogen. The sulphonic acid (para) is employed for the preparation of the tartrazine series of dyes.

Phenylhydrazides.—The acetyl-derivative of phenylhydrazine, $C_6H_5.HN.NH(C_2H_3O)$, which may be regarded as acetphenylhydrazide, has powerful antipyretic properties, and has been introduced into German pharmacy under the name of "hydracetin." The same substance is said to be the active ingredient of the preparation known as "pyrodine" (*Pharm. J.* [iii], **19**, 425, 508, 1049). Both substances seem to be uncertain in their action and dangerous in use; in fact, hydracetin is reported by Renvers to be a direct blood-poison, the antithermic properties of which are really due to destruction of the red corpuscles.

"Orthine" is the name given by R. Kobert to orthohydrazino-parahydroxy-benzoic acid:

$$C_6H_3 \begin{cases} (OH)^{(1)} \\ (HN.NH_2)^{(2)} \\ (CO.OH)^{(4)} \end{cases}$$

The free base is very unstable; but the hydrochloride is stable, reduces the persalts of the heavy metals, and possesses a marked antiseptic action.

Phenylhydrazine in aqueous solution reacts very readily with the hydroxy-acids of the sugar group (*e. g.*, gluconic and galactonic acids, $C_5H_6(OH)_5.COOH$; arabinose-carboxylic acid, $C_6H_{12}O_7$) with elimination of water, to form crystalline phenylhydrazides, R.CO.HN.-$NH(C_6H_5)$. They are prepared by treating a 10% solution of the acid or its lactone with a moderate excess of phenylhydrazine and an equal quantity of 50% acetic acid, and heating the mixture to 100° for 80 to 120 minutes. The hydrazide sometimes crystallises from the hot solution, but more usually separates on cooling. Any free mineral acid should be neutralised by sodium carbonate before adding the phenylhydrazine, and bromides, chlorides and sulphates should be eliminated by means of lead acetate. If a sugar be present, the osazone formed can usually be separated from the hydrazide by crystallisation from hot water. The products are beautifully

crystalline, those derived from monobasic acids being but little soluble in cold, and only with difficulty soluble in hot water, while those from polybasic acids (*e. g.*, saccharic, metasaccharic, and mucic) are still less readily soluble. The compounds from isomeric acids usually present a close resemblance in their physical properties, but the acids from which they are derived can be regenerated (in a pure state) by boiling the hydrazide for half an hour with 30 volumes of 10% baryta water, which treatment hydrolyses them completely. From the product, the phenylhydrazine is extracted by agitation with ether, and the aqueous liquid, with any precipitate which may have been formed, is boiled and treated with sulphuric acid in quantity sufficient to precipitate the barium as $BaSO_4$. The filtered liquid yields the free acid or lactone on evaporation (Fischer and Passmore, *Ber.*, 1889, **22**, 2728).

The hydrazides are colourless and readily hydrolysed by alkalies and baryta. They can be readily distinguished from the hydrazones by the reddish-violet coloration they give when dissolved in strong sulphuric acid and treated with a drop of ferric chloride solution.

Detection of Phenylhydrazine

Simon (*Compt. Rend.*, 1898, **126**, 483 and *Bull. Soc. Chim.*, 1898, **19**, 299) devised the following test for detecting phenylhydrazine and its substituted derivatives which behave in the same way: The test is capable of detecting phenylhydrazine in a solution of 1 in 50,000. The solution is momentarily warmed with a few drops of aqueous trimethylamine and several drops of a solution of sodium nitroprusside are then added. A colour varying from blue to green is produced, which becomes more pronounced on adding a little concentrated potassium hydroxide solution. If a little acetic acid be added, either before or after the potassium hydroxide, the colour is of a sky-blue shade. Added in excess, acetic acid causes the colour to disappear. Ether and alcohol do not affect the test, but chloroform and benzene interfere with it. Acetone gives its own coloration (the red of Legal's test). Mineral or organic acids retard the production of colour until after the addition of potassium hydroxide. Ammonia does not interfere with the test. On heating the blue coloured liquid the tint becomes red when potassium hydroxide is present, and clear yellow when it is absent. Simon's reaction is not given by hydrazones and seems to be characteristic of phenylhydrazine and its

substituents. Negative results are given by formyl and benzoylphenylhydrazine (*i. e.*, substituted compounds of the type Ph.NH.-NH.Ac.).

The blue colour is easily distinguishable from that given by aldehyde with the same reagents by its persistence in the presence of potassium hydroxide, ammonia and acetic acid.

Rimini (*Ann. Farm.*, 1898, 102) states that *pure* trimethylamine does not give the above reaction, but that it is due to the presence of formaldehyde, which can be substituted with advantage in the test (see Vol. 1).

Estimation of Phenylhydrazine

Causse's method is as follows (*Compt. Rend.*, 1898, **125**, 712):—It is based on the reduction of arsenic acid according to the equation $As_2O_5 + C_6H_8N_2 = N_2 + H_2O + C_6H_6O + As_2O_3$. The solutions required are: 1. Arsenic acid solution prepared by dissolving on a water bath 125 grm. of arsenic acid in a mixture of 450 c.c. of water and 150 c.c. of hydrochloric acid, then when cold making the solution up to 1000 c.c. with glacial acetic acid; 2. N/10 iodine solution; 3. 200 grm. of sodium hydroxide (free from sulphides) dissolved in 1 litre of water; 4. cold saturated solution of sodium hydrogen carbonate. 0.2 grm. of the sample of phenylhydrazine or its hydrochloride is placed in 500 c.c., 60 c.c. of the arsenic acid solution added and the liquid boiled under a reflux condenser, using a spiral of platinum wire to prevent bumping. When action has ceased, that is, after about 40 minutes, the liquid is cooled, 200 c.c. of water are added and then sodium hydroxide until the liquid is alkaline, and finally a drop or two of hydrochloric acid. 60 c.c. of the sodium hydrogen carbonate are then added and the arsenious acid estimated by iodine solution and starch. The method can be applied also to aromatic phenylhydrazones, but in the case of fatty phenylhydrazones the aldehyde should be removed before titration, on account of its action on arsenic acid.

Knecht has shown that, although phenylhydrazine is not acted upon by titanous chloride, it can be accurately estimated with this reagent by an indirect method. This is particularly useful, as it opens up a direct method for estimating carbohydrates and their osazones (*cf. J. Chem. Soc.*, 1924, **125**, 1537 and 2009). The estimation of phenylhydrazine is based on the fact that 1 mol. of methylene

blue requires for its reduction to the leuco-compound exactly 1 mol. of phenylhydrazine. By adding the phenylhydrazine to an excess of methylene blue, boiling for about a minute in a current of carbon dioxide, and titrating back the excess with titanous chloride accurate results are obtained.

$$C_{16}H_{18}N_3ClS + C_6H_8N_2 = C_6H_6 + C_{16}H_{20}N_3ClS + N_2.$$

Standard titanous chloride solution is prepared by mixing 50 c.c. of commercial 20 per cent. titanous chloride solution with 50 c.c. of hydrochloric acid, and diluting to 1 litre with boiled distilled water; the solution is standardised against 0.1 N potassium dichromate.

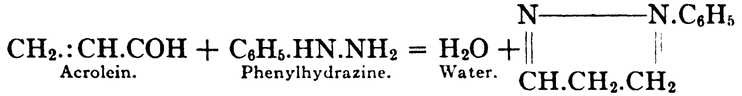
Hydrazones.—Phenylhydrazine behaves in a highly interesting manner with aldehydes and ketones, with which it reacts, with elimination of water to form hydrazones. Most of the substances of this class are solid and crystalline, and therefore well suited for the recognition of the aldehydes or ketones producing them. The action is general for substances containing the carbonyl group, CO, but is sometimes complicated by the presence of other reactive groups. Thus compounds containing the α-ketone-alcohol-group—CH(OH).-CO—react in the cold with only 1 molecule of phenylhydrazine to form colourless compounds containing the group CH(OH).C.-($N.NHC_6H_5$).

Osazones.—When the compound thus formed is heated with excess of phenylhydrazine, the alcohol group undergoes oxidation, reacting at the same time with a second molecule of phenylhydrazine and giving rise to a yellow compound containing the complex group—$C(N.NHC_6H_5).C(N.NHC_6H_5)$. Compounds of this kind, in which 2 hydrazine residues are attached to 2 contiguous carbon atoms, are called osazones, and may be obtained directly by the action of phenylhydrazines on the di-ketones. They are of interest in connection with the carbohydrates, which may frequently be recognised by means of their characteristic osazones. A solution of phenylhydrazine hydrochloride containing sodium acetate can be used for the detection of sugar in urine.

Osazones may be estimated, without necessarily being isolated, by the titration method of Knecht (*loc. cit.*). To a solution of the ozazone containing about 0.03 grm. are added 2 c.c. of saturated solution of sodium tartrate and 50 c.c. of standard titanous chloride solution. The mixture is boiled in a atmosphere of carbon dioxide

for six minutes, excess of hydrochloric acid is added and the titanous chloride titrated back with Crystal Scarlet solution. The reduction takes place in accordance with the equation (for glucosazone) $C_{18}H_{22}O_4N_4 + 6H + H_2O \rightarrow C_6H_{13}O_5N + NH_3 + 2C_6H_5.NH_2$. The crystal scarlet is standardised against the titanous chloride solution.

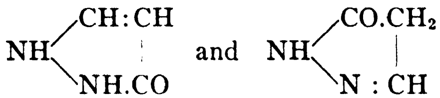
Pyrazolines.—An unsaturated hydrocarbon group (*e. g.*, allyl, C_3H_5), if contiguous to the carbonyl group, may also react with phenylhydrazine:



Acrolein. Phenylhydrazine. Water. Phenyl-pyrazoline.

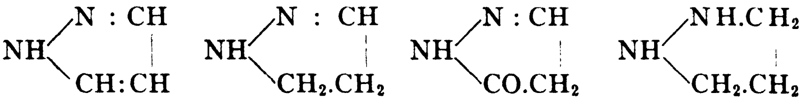
Pyrazolones

The pyrazolones are derivatives of a substance of the formula $C_3H_4N_2O$, which exists in two varieties: 3-pyrazolone and 5-pyrazolone, of which the former is only known in its derivatives. These have the formulæ:



3 Pyrazolone 5 Pyrazolone

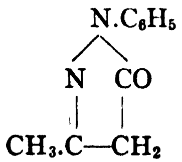
The relationship of pyrazolone to pyrazole, pyrazoline, and pyrazolidine is shown by the following formula:



Pyrazole. Pyrazoline. Pyrazolone. Pyrazolidine.

Phenylpyrazolones. Antipyrin

1:3 Phenyl-methylpyrazolone, $C_{10}H_{10}ON_2$;



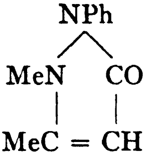
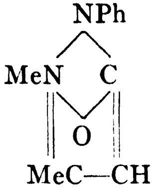
This compound is of interest, as it condenses with various aldehydes and ketones and with diazotised substances forms azo-dyes. It is an intermediate compound in the manufacture of antipyrin. When phenylhydrazine is added to ethyl aceto-acetate, $CH_3.CO.CH_2.$-$CO.O(C_2H_5)$, the two substances interact in the cold, with elimination of water, to form $CH_3.C(N.NHPh)CH_2.CO.O(C_2H_5)$.[1] On heating, the hydrazone thus formed splits up into alcohol and phenyl-methyl-pyrazolone, a substance which was originally regarded by its discoverer, Knorr, as a methyl-oxyquinizine.

To prepare 1:3-phenyl-methylpyrazolone, 100 parts of phenylhydrazine are added to 125 of ethyl aceto-acetate, the water which forms is separated, and the oily product is heated for 2 hours on a water-bath, until a portion is found to solidify on cooling, or on the addition of ether. The warm mass is poured into and stirred with ether, which removes colouring matter, and the white crystalline product is washed with ether, and dried at 100°. The yield is quantitative and the product pure. It is almost insoluble in cold water, ether, and petroleum spirit, more readily in hot water, and easily in aclohol. It crystallises from hot water or alcohol in hard brilliant prisms and melts at 127°.[2] The *hydrochloride*, $C_{10}H_{10}ON_2,HCl + H_2O$, melts at 96°, and the *platinichloride*, $(C_{10}H_{10}ON_2)_2H_2PtCl_6 + 4H_2O$, in prisms melting at 100°. Phenyl-methylpyrazolone yields crystalline precipitates with salts of many of the heavy metals. With silver nitrate an aqueous solution gives crystals of $C_{10}H_9AgON_2 + C_{10}H_{10}ON_2$. The ultramarine cobalt compound and the orange-yellow uranium salt are especially characteristic.

[1] **Antithernim.**—When an aqueous solution of lævulinic acid (aceto-propionic acid), $CH_3.CO.CH_2.CH_2.COOH$, is added to an equivalent amount of phenylhydrazine, dissolved in dilute acetic acid, a yellow precipitate is produced of the hydrazone, $CH_3C(N.$-$NHPh).CH_2.CH_2.COOH$. When recrystallised from alcohol, this forms large colourless, odorless crystals of a slight bitter taste, melting at 108°, and nearly insoluble in water, but soluble in alcohol, ether, and dilute acid. It has met with a limited application as an antipyretic under the name of antithermin and also in cases of phthisis and Bright's disease. It is decomposed by alkalies with liberation of phenylhydrazine, to which fact it probably owes its physiological activity.

[2] When a mixture of phenyl-methylpyrazolone and phenylhydrazine is heated to boiling, bisphenyl-methylpyrazolone, $C_{20}H_{18}O_2N_4$ is formed. Heated with methyl alcohol or methyl iodide it yields diantipyrin, $C_{22}H_{22}O_2N_4$, melting at 245°, and distinguished from antipyrin by its sparing solubility in water and the m. p., of its picrate (161°). When the compound $C_{20}H_{18}O_2N_4$ is treated in alkaline solution with excess of sodium nitrite, and the mixture poured into dilute sulphuric acid, *pyrazole-blue*, $C_{20}H_{16}O_2N_4$, separates in flocks. When crystallised from chloroform it forms blue needles, insoluble in water, dilute acids, and alkalies, and only sparingly soluble in alcohol and ether. Its solutions in chloroform and strong sulphuric acid has an indigo blue colour.

1-Phenyl-2:3-Dimethylpyrazolone. Antipyrin. Phenazone.

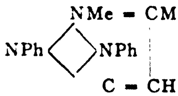
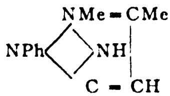
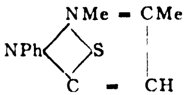
$C_{11}H_{12}ON_2$ =  (Knorr) or  (Michaelis[1])

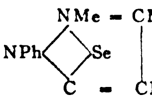
When 1-phenyl-3-methylpyrazolone is heated with methyl iodide, a further action takes place, with formation of phenyl-dimethylpyrazolone, a substance known generally as "antipyrin," less commonly as "analgesin," or "anodynin" and in the *British Pharmacopœia* (1914), *phenazone*. It is official in the *German Pharmacopœia* under the name of *Antipyrinum*.

Antipyrin is prepared by heating equal parts of phenyl-methylpyrazolone, methyl iodide, and methyl alcohol to 100° in a closed vessel. The dark product is decolorised by boiling with sulphurous acid, the alcohol distilled off, and the residue shaken with strong sodium hydroxide, when the base separates as a heavy oil. This is separated and treated with ether, in which it is sparingly soluble. On separating the ether and evaporating off the solvent, the antipyrin is obtained as a mass of crystals, which are purified by recrystallisation from toluene.

Antipyrin forms small, lustrous, rhombic needles or plates, which are odourless, but have a somewhat bitter taste. When perfectly anhydrous it melts at 111°–113° (*British Pharmacopœia*,) 114° (*German Pharmacopœia;*) 111°–113° (*United States Pharmacopœia*, 9th Rev.), but on exposure to air takes up a small proportion (0.6%) of water, and in that state melts at 105°–107°. The hygroscopic water may

[1] Michaelis (*Annalen*, 1902, **320**, 1) considers that the formula given, which represents antipyrin as a 2:5-pyrazole, is more in accord with its properties than the customary formula which represents it as a pyrazolone. Michaelis' formula best explains the formation and properties of *anilopyrin*,

 *iminopyrin*,  *thiopyrin*, 

and *selenopyrin*,  (Compare *Ber.*, 1899, **32**, 2398; 1901, **34**, 723.)

be driven off by exposing the substance to a temperature of 100°, when the original m. p. is restored.

Antipyrin is soluble in about its own weight of cold water, and in less than half its weight of boiling water. It dissolves in twice its weight of absolute alcohol, but in little more than its own weight of rectified spirit (1.3 part of alcohol, *United States Pharmacopœia*, xth Rev.) Antipyrin is soluble in an equal weight of amyl alcohol, and in one and a half times its weight of chloroform, but requires about 50 parts of ether for solution (43 parts of ether at 25°, *United States Pharmacopœia*), is difficultly soluble in benzene, and nearly insoluble in petroleum spirit.

On adding strong sodium hydroxide to an aqueous solution of antipyrin, the base separates as a milky precipitate, which speedily collects into oily globules. On adding a little ether, these immediately solidify to white crystals without appreciably dissolving, but they dissolve instantly on adding chloroform (J. C. Waterhouse).

An aqueous solution of antipyrin exhibits no alkaline reaction with litmus or phenolphthalein, but destroys the red colour of an acidified solution of methyl orange. Free antipyrin may be estimated with accuracy by titration in aqueous or alcoholic solution with Methyl orange.

Antipyrin is a strong monacid base. Its salts, most of which are soluble, do not readily crystallise, with the exception of the *picrate* (m. p. 188°); the *ferrocyanide* $(C_{11}H_{12}ON_2)_2,H_4Cfy$, which forms a crystalline precipitate; the *platinichloride*, $(C_{11}H_{12}ON_2),H_2PtCl_6 + 2H_2O$, which forms yellowish-red prisms m. p. about 200°; and the *salicylate* (page 321).

When antipyrin is heated with hydrochloric acid under pressure to 200°, it suffers complete decomposition, yielding much aniline and a small quantity of methylamine, besides other products. On distillation with zinc dust it yields benzene, aniline, a base boiling at 86° to 87°, and other products.

Antipyrin is unchanged by treatment with reducing agents in the wet way, but with oxidising agents it gives a series of interesting reactions (Gay and Fortuné, *Pharm. J.* [iii], **18**, 1066). Thus when boiled with potassium chlorate and hydrochloric acid, antipyrin gives a reddish-yellow liquid, which on cooling deposits bright-red oily globules which dissolve in chloroform with greenish-yellow

colour. A solution of bleaching powder produces no change in the cold, but, on heating, a brick-red precipitate is formed, and the liquid is coloured yellow. Sodium hypochlorite is said to give the yellow coloration on heating without any precipitate being formed. Chlorine water produces no change, and bromine water a light yellow precipitate, dissolving on heating. Potassium dichromate and permanganate are reduced by acid solutions of antipyrin.

Tannic acid dissolved in water gives a copious white precipitate of tannate with solution of phenazone.

When a solution of iodine in potassium iodide is added to a solution of antipyrin, a precipitate is formed which disappears on agitation, leaving the solution colourless; but on further addition of the reagent, a permanent brick-red precipitate is produced, perceptible in a dilution of 1 in 20,000. According to Manseau (*Pharm. J.* [*iii*], **20**, 162), the point at which a permanent precipitate is formed is perfectly definite, and he suggests that the purity of a sample can be ascertained by titration with a standard solution of iodine. Millard and Stark (*Pharm. J.* [iii], 20, 863) find that the point of permanent precipitation depends to a marked degree on the dilution of the antipyrin solution. Thus in a 1% solution, 1 grm. of antipyrin gives a permanent precipitate after the addition of 3.9 c.c. of N/10 iodine, while with twice the volume of water 7.2 c.c. are required. The authors state that more concordant results are obtainable by using starch as an indicator. They dissolve 0.5 grm. of the sample of antipyrin in 200 c.c. of water, add plenty of starch solution, and then drop in N/10 iodine solution gradually until a distinct blue coloration is obtained, which does not disappear on vigorously shaking or stirring the mixture. (*Iodo-antipyrin.*)

An acid solution of mercuric nitrate gives a white precipitate with a solution of antipyrin. 2 c.c. of Millon's reagent and 4 c.c. of a 1% (neutral) solution of antipyrin give a white precipitate in a yellow liquid; in a solution acid with hydrochloric acid, a yellow precipitate in an orange-yellow liquid, the precipitate eventually becoming red. In a solution 10 times more dilute a yellow precipitate and green liquid results, and in an acid solution of 1 part of antipyrin in 20,000 a white precipitate and yellow liquid. 1 c.c. of a saturated solution of mercurous nitrate added to twice its volume of a 1% solution of antipyrin gives a yellow precipitate floating on a blood-red liquid.

If antipyrin be heated with strong nitric acid till action commences, and the liquid then allowed to cool, a fine purple coloration is produced; on adding water a violet precipitate is thrown down, and the filtered liquid is purple-red.

Iso-nitroso-antipyrin.—Several of the foregoing indications are probably due to the presence of nitrous acid, which (if added in the form of red fuming nitric acid) gives with a 1% solution of antipyrin a beautiful green coloration, still perceptible when diluted to 1 in 20,000; when the liquid is heated it becomes purple-red. In strong solutions a copious formation of small, green, needle-shaped crystals occurs. These consist of iso-nitroso-antipyrin, $C_{11}H_{11}(NO)ON_2$. and are best obtained by adding a solution of sodium nitrite to a solution of antipyrin in acidified water. The liquid at once becomes bluish-green in colour, and an abundant formation of crystals speedily occurs. These may be washed with cold water, and dried at the ordinary temperature.[1] Nitroso-antipyrin explodes when heated to about 200°, is nearly insoluble in water and dilute acids, soluble in alkalies and in acetic acid, moderately soluble in alcohol, and sparingly in chloroform and ether. By treatment with zinc and acetic acid it is converted into an oily base.

The green coloration of antipyrin with nitrous acid is delicate and, to a certain extent, characteristic, but is common to all pyrazolones. A. C. Stark recommends that the test should be applied by dissolving potassium nitrite in a test-tube in a little water, adding excess of strong sulphuric acid, and then filling the tube with the liquid to be tested.

Antipyrin dissolves without colour in pure anhydrous ethyl nitrite, but a green colour is immediately developed on addition of water. When antipyrin is added to spirit of nitrous ether containing free acid, the mixture rapidly acquires a dark-green tint, and green needles of iso-nitroso-antipyrin separate. The action (which does not occur if any free acid be neutralised by potassium hydrogen carbonate) derives practical importance from the fact that spirit of nitrous ether and antipyrin are not infrequently dispensed in conjunction. A mixture of the kind is alleged to have been fatal to the patient, but it is very doubtful if the nitroso-derivative of antipyrin was the cause of death; for direct administration of the compound

[1] The liquid filtered from the crystals gradually changes colour from green to brown, and after standing for some hours is found to smell of hydrocyanic acid, but the quantity of this substance formed appears to be very minute (Wood and Marshall, *Pharm. J.* [iii], 19, 806).

to a small rabbit, both hypodermically and by the stomach, in doses commencing at ½ grain, and gradually increased to 4 grains, produced no perceptible toxic effect (*Pharm. J.* [*iii*], **18**, 1085). Similar experiments have been made on dogs (*Pharm. J.* [*iii*], **19**, 807).

Antipyrin gives a very delicate and characteristic reaction with ferric chloride, which, in a 1% solution, produces a blood-red coloration. The reaction is still very distinct in a solution of 1 in 2000 and perceptible at a dilution of 1 in 50,000. The red coloration is destroyed by excess of mineral acids. The reaction is at once given by urine containing antipyrin.

On mixing cold aqueous solutions of antipyrin and mercuric chloride, a white precipitate is formed. On boiling the liquid this disappears, but on continued boiling a brown resinous substance is deposited, which, when separated, is found to be soluble in hot alcohol and in nitric acid, and is coloured scarlet by concentrated sulphuric acid.

Antipyrin behaves in the general manner of alkaloids. Thus, in acid solutions it gives a yellowish-white precipitate with Mayer's reagent, and the same with Marmé's test (potassium-cadmium iodide); a green precipitate changing to orange-red with bismuth potassium iodide; an abundant reddish-yellow precipitate with Nessler's reagent; a white with sodium phosphomolybdate and an abundant white precipitate with tannin.[1]

Pharmacopœia Requirements

The British Pharmacopœia (1914) gives the following: m. p. 111°–113°, aqueous solution neutral to litmus; a 1:100 aqueous solution responds to the following tests: mixed with an equal volume of nitric acid it assumes a yellow colour passing to crimson on warming; 2 c.c. are coloured green by 2 drops of priming nitric acid and the colour is changed to red by boiling with an additional 3 or 4 drops of the priming nitric acid; 12 c.c. remain nearly colourless on addition of 0.1 grm. of sodium nitrite, but turn a deep green on the further addition of 1 c.c. of diluted sulphuric acid. 1 c.c. diluted with 4 c.c. of water is coloured deep red by solution of ferric chloride, the colour

[1] The reactions described in the text sufficiently indicate the pharmaceutical preparations with which antipyrin is incompatible. Thus it should not be dispensed in a mixture with nitric acid, nitrites, chloral hydrate, sod sodiulim salicylate, carbolic acid, tannin, iodine, mercuric chloride, salts of iron, permanganates, or tinctures of infusions of catechuh, cinchona, roses, galls, rhubarb, etc. (see Millard and Stark, *Pharm. J.* [*iii*], **20**, 860).

being nearly discharged by excess of diluted sulphuric acid. An aqueous solution (1:20) gives with solution of mercuric chloride a white precipitate which disappears on boiling, but reappears on cooling. Aqueous solution not affected by hydrogen sulphide. No appreciable ash.

The requirements of the *United States Pharmacopœia*, Tenth Revision, are as follows:

M. p. 111°–113°. Must not leave not more than 0.1 per cent. of ash on ignition. If to an aqueous solution, tannic acid (T. S.) is added, an abundant white ppt. is formed. If 0.1 grm. of sodium nitrite and 12 c.c. of an aqueous solution of antipyrin (1 in 100) be mixed, a nearly colourless liquid is obtained which upon the addition of 1 c.c. of dilute sulphuric acid develops a deep green colour (formation of iso-nitroso-antipyrin).

If to 2 c.c. of a dilute aqueous solution of antipyrin (1 in 1000) 1 drop of ferric chloride T. S. be added, a deep red colour is produced which upon the addition of 10 drops of sulphuric acid is changed to light yellow.

The antipyrin must be soluble in its own weight of cold water, the solution being colourless or not more than slightly yellow when viewed transversely in a tube having a diameter of about 20 mm. The aqueous solution (1:20) must be neutral to litmus.

According to the *German Pharmacopœia*, the solution of antipyrin in 2 parts of water should be neutral, free from acrid taste, and not changed by hydrogen sulphide water. A 2% solution should give a white precipitate with tannin; and on addition of 2 drops of fuming nitric acid to 2 c.c. of the solution, a green coloration should occur, changed to red on boiling and adding another drop of nitric acid.[1] 2 c.c. of a 0.2% solution gives a deep red colour with a drop of ferric chloride solution, changed to bright yellow on adding 10 drops of sulphuric acid. The m.p. is put at 110–112°.

The *Japanese Pharmacopœia* is similar to the German in its tests, but gives m.p. 110–113°.

Antipyrin has now an establised position and wide application in medicine. Although originally introduced as a febrifuge, it is taking a still higher place as an anodyne. Given in 10 to 20 grain doses in

[1] This red coloration is said by Sperling (*Chem. Centr.*, 1906, I, 1118) to be due to nitro-antipyrin. It is not always distinctly apparent and sometimes a brown coloration is obtained. Antipyrin and all its derivatives, except aminoantipyrin, give the following reaction: 2 drops of fuming nitric acid are added to 2–3 c.c. of a 1% solution in water and then 5% of conc. sulphuric acid are cautiously added. A cherry-red ring is formed at the surface of contact and when the layers are mixed the colour permeates the mixture.

cases of bilious and nervous headache, it often effects a remarkably rapid and perfect cure. It has been usefully injected hypodermically in 8-grain doses as a substitute for morphia; and for the relief of pain in acute and chronic gout, neuralgia, sciatica, etc. The subcutaneous injection of antipyrin is said not to be followed by drowsiness, vomiting, or excitement. It is stated to be almost a specific in puerperal fever. It has been found valuable as a hæmostatic, and has proved successful in some cases of sea-sickness, but by no means invariably. Antipyrin causes an almost immediate reduction in the temperature of the body (apparently from its influence on the brain-centres regulating the temperature), the effect continuing from 4 to 6 hours. It induces sweating and feeble pulse, and in excessive doses, or even small doses in certain cases, an eruption resembling nettle-rash, occasionally with vomiting and collapse.[1] Atropine has been found to act promptly as an antidote.

Antipyrin may be detected in the urine for 18 to 24 hours after it is taken by the stomach, but can be detected only for a few hours in the different organs. It has been detected, after putrefaction for a fortnight, in animals killed within 2 hours after its administration, either by the stomach or hypodermically.

Antipyrin is readily extracted from animal matters by rendering the liquid ammoniacal and agitating it with chloroform or amyl alcohol.

Steensma (*Pharm. Weekblad.*, 1907, **44**, 1066) recommends *p*-dimethylaminobenzaldehyde as a means of detecting antipyrin. The reagent is prepared by diluting a solution of 1 grm. of the aldehyde in 5 c.c. of 25% hydrochloric acid to 100 c.c. with absolute alcohol. When a small portion of this solution in presence of a trace of antipyrin is evaporated in a porcelain dish to dryness on the water-bath, a light red stain is left. The test serves to detect 0.001 mgrm. of antipyrin (*cf.* Lander and Winter, *Analyst*, 1913, **38**, 97). Aqueous solutions should be extracted with chloroform, the solvent evaporated and the residue dissolved in the reagent. It may also be detected by the U. S. P. ferric chloride test.

Estimation of Antipyrin

1. **Kippenberger's Iodometric Method.**—On adding a solution of iodine or an iodide to aqueous solutions of antipyrin, acidified or

[1] The administration of antipyrin is unsafe when the heart is weak. A case where severe symptoms were produced by a dose of 1 grm. has been recorded by Schwabe (*Pharm. J.* [iii], **20**, 1059).

not, a brown tarry, non-crystallisable mass of the composition $C_{11}H_{12}ON_2$, HI, I_2, separates. Advantage may be taken of this fact to separate antipyrin from phenacetin, sulphonal, acetanilide and aniline salts if acid be present, hydrochloric acid being most suitable. The process is carried out as follows:

To the solution of antipyrin (as concentrated as possible) contained in a stoppered flask, a solution of iodine is added, made by mixing 100 c.c. of an N/20 iodine solution, containing 10 or 20 grm. of potassium iodide per litre, with about 4 c.c. of hydriodic acid of sp. gr. 1.7 (52% HI). *Only a small excess of iodine solution is added*, and the flask then well shaken until the liquid becomes clear, the precipitate adhering to the walls of the flask. The liquid is filtered through a small asbestos filter into a dry burette and in an aliquot portion of the filtrate the iodine is estimated by N/20 thiosulphate. 21.3 c.c. of N/20 iodine = 0.1 grm. of antipyrin. The error due to the solubility of the periodide is generally negligible, but may be allowed for by standardising the iodine solution against a solution of antipyrin of known strength.

Salipyrine and other antipyrin salts may be estimated in the same way.

The iodometric method may conveniently be applied volumetrically by adding to the aqueous solution an excess of about 1 grm. of sodium bicarbonate and excess of 0.1 N iodine solution; after an hour the mixture is acidified with acetic acid, 10 c.c. of chloroform are added, and the excess of iodine is titrated with 0.1 N thiosulphate solution. Under these conditions two atoms of iodine combine with one molecule of antipyrin. (Bougault, *J. Pharm. Chim.*, 1917, **15**, 339.)

2. **Picric Acid Method** (Lemaire, *Pharm. J.*, 1905, **74**, 13).—A known volume of the solution containing antipyrin is treated with a definite excess of an N/20 solution of picric acid and the sparingly soluble picric filtered off after standing. The free picric acid is estimated in an aliquot portion of the filtrate by titration with N/10 sodium hydroxide, using phenolphthalein as indicator. 1 mol. of antipyrin (188) combines with 1 mol. of picric acid (229).

W. O. Emery and S. Palkin (*J. Ind. Eng. Chem.*, 1914, **6**, 751) give the following methods of estimating antipyrin either alone or in presence of other substances.

I. *Alone or free from substances yielding a derivative capable of being extracted with chloroform.*

A quantity of the sample containing not more than 0.25 grm. of antipyrin is dissolved in 20 c.c. of water and treated with 5 c.c. of alcohol-free chloroform, 0.5 grm. of sodium hydrogen carbonate and a slight excess of iodine (15–20 c.c. of N/5 solution); after vigorously agitating at intervals during 5 minutes, the free iodine is removed by adding thiosulphate and the iodo-antipyrin extracted by shaking thrice with 25 c.c. of chloroform each time. The chloroform extract is washed with water, filtered, evaporated and the residue dried during 30 minutes at 110° and weighed. The weight multiplied by 0.5992 gives the quantity of antipyrin.

II. *When antipyrin is mixed with acetanilide, phenacetin, sulphonal or other substances which do not give an iodine derivative insoluble in aqueous acid.*

The sample (containing not more than 0.25 grm. of antipyrin) is dissolved in 50 c.c. of water and shaken well with 20 c.c. of concentrated hydrochloric acid and 50–60 c.c. of N/10 iodine; after 3 hours, the clear liquid is decanted through a filter of glass wool and asbestos, and the tarry precipitate of antipyrin periodide washed eight to nine times by decantation with 5% hydrochloric acid, and dissolved in 50 c.c. of methyl alcohol free from ethyl alcohol and acetone. The solution is treated with 5 c.c. of sodium hydrogen carbonate solution and 50 c.c. of water, shaken for 5 minutes, whereby the periodide is converted into iodo-antipyrin, the excess of iodine is removed by thiosulphate, and the iodo-antipyrin extracted by shaking three times with 40 c.c. of chloroform each time and estimated as described in I.

Detection of Adulterants in Antipyrin

The following methods are given by Raikow and Schtarbonow (*Oester. Chem. Zeit.*, 1900, **3**, 125).

Acetanilide (antifebrin) and phenacetin (*p*-acetamino-phenetole) can be detected by boiling the antipyrin with concentrated phosphoric acid, the two anilides yielding acetic acid under these conditions, which can be recognised by its smell. Antipyrin gives a yellow colour with phosphoric acid which gradually changes to brownish-yellow; acetanilide gives a faint yellow colour which becomes brown on boiling. With phenacetin the solution is first rose coloured, then brownish red, changing through reddish-violet to violet, bluish-green

to a dirty green. The appearance of a violet coloration is specially characteristic of phenacetin.

Acetanilide and phenacetin are distinguished by their different behaviour on hydrolysis with potassium hydroxide. A few grm. of the substance are heated with 2–4 c.c. of conc. aqueous potassium hydroxide in a test-tube, fitted with a rubber stopper through which passes a glass tube connected with a second test-tube containing 1–3 c.c. of a clear solution of calcium hypochlorite (bleaching powder). If acetanilide is present in the antipyrin the first drops of the distillate produce the well-known violet coloration characteristic of aniline. In the absence of acetanilide and presence of phenacetin the first drops give no coloration but subsequently a brick-red turbidity due to phenetidine is produced. Finally an amorphous red substance separates on the surface of the liquid, which becomes clear yellow in colour. If the receiving test-tube be changed when both acetanilide and phenacetin are present, the two indications may be observed successively. On boiling a mixture of phenacetin and antipyrin with potassium hydroxide the distillate does not give the above described red coloration characteristic of phenacetin, but the solution becomes yellowish-green and then yellowish-grey. With antipyrin alone the bleaching powder solution remains colourless.

Exalgin (methylacetanilide) when boiled with phosphoric acid is readily hydrolysed, giving acetic acid and the phosphoric acid becomes coloured intense golden yellow. On boiling with potassium hydroxide, methylanilkne distils over and collects in oily drops on the surface of the calcium hydrochlorite: a green colour is produced which becomes greyish-green and finally a dirty green.

Salipyrine (Antipyrin salicylate), $C_{11}H_{12}ON_2$, $C_7H_6O_3$.—If salicylic acid be gradually added to a dilute boiling solution of antipyrin, antipyrin salicylate separates as a yellowish oil. The compound can be more conveniently prepared by heating equivalent proportions of antipyrin and salicylic acid with a little water to 90°, or by shaking together an aqueous solution of antipyrin with an ethereal solution of salicylic acid, when the salt separates in fine crystals. Antipyrin salicylate melts at 91–92°, and decomposes at a somewhat higher temperature, dissolves in 250 parts of cold water and in 25 parts hot, and readily in alcohol, ether, chloroform, and carbon disulphide. The aqueous solution is faintly acid in reaction, and has a sweet taste and bitter after-taste. It gives a

violet-red coloration with ferric chloride, and green with nitrous acid. It contains 57.7 per cent. of antipyrin with 42.3 per cent. of salicylic acid and may be assayed by adding to it excess of N sodium hydroxide, then shaking out the base with chloroform, and titrating the excess of sodium hydroxide with N sulphuric acid, phenolphthalein being used as indicator; the base and acid may of course be subsequently identified by their melting points. Antipyrin salicylate has been employed with favourable results in medicine under the name of "salipyrine." A mixture of antipyrin and sodium salicylate gradually changes to an oily liquid on exposure to air. The change, which does not occur in a closed bottle, appears to be simply due to absorption of moisture by the salicylate and the solution of the antipyrin in the water thus absorbed. Antipyrin salicylate is official in the *Japanese Pharmacopœia.*

Resalgin (Resopyrin).—Antipyrin becomes pasty when mixed with β-naphthol, and appears to form a compound with phenol. Under the name of "resopyrin," Portes has described a compound obtained by mixing solutions of molecular proportions of resorcinol and antipyrin. It crystallises in oblique rhombic prisms, insoluble in water but soluble in alcohol.

Iodoantipyrin, $C_{11}H_{11}ION_2$.—is a compound formed by the action of iodine on antipyrin in the presence of sodium acetate; it exists as colourless needles, having m. p. 160°. This substance is sometimes used in medicine, as it combines the properties of iodine with those of an antipyretic. The formation of iodine compounds of antipyrin gives a method for its estimation cf. p. [314].

Hypnal (Chloral-antipyrin), $C_{11}H_{11}(C_2H_2Cl_3O)ON_2$.—When dilute solutions of chloral hydrate and antipyrin are mixed no perceptible reaction occurs, but on concentrating the liquid, or on mixing strong solutions of the 2 substances, a separation of oily globules takes place, and these immediately or gradually change to a mass of crystals of chloral-antipyrin. The same substance may be obtained by heating molecular proportions of chloral hydrate (165.5 parts) and antipyrin (188 parts) to 110–115°. The action consists in elimination of water and substitution of the group $CCl_3.CH(OH)$ for one of the hydrogen atoms of the antipyrin; but whether the replaced atom is one of those of the methyl groups, or the hydrogen atom of the CH group, is not definitely decided (compare *Pharm. J.* [*iii*], **20**, p. 862 with p. 889).

Chloral-antipyrin crystallises from alcohol in hard scales and from water in transparent rhombs. It melts at 67–68°, is almost odourless, and has a saline taste with an after-taste suggestive of chloral. It is only slightly soluble in cold alcohol, ether, and chloroform, but somewhat more soluble in boiling alcohol, and is dissolved by about 8 parts of warm water. The solution reduces Fehling's solution on warming, gives the blood-red reaction of antipyrin with ferric chloride, and yields chloroform when heated with dilute alkali hydroxide. When chloral-antipyrin is kept in a melted state for some time, it deposits crystals of a *dehydration compound*, which is insoluble in water, melts at 186–187°, and gives no colour-change with ferric chloride. According to Reuter (*Pharm. J.* [iii], **20,** 602) chloral-antipyrin is physiologically inert, but Bardet found doses of 1 grm. to induce sleep as readily as chloral hydrate, while in cases of insomnia caused by pain it seemed to have the same anodyne effect as antipyrin. Schmidt found the monochloral derivative to have more decided soporific effect and a less deleterious influence on the circulation than antipyrin.

Bichloral-antipyrin is obtained by heating antipyrin with excess of a strong solution of chloral hydrate, when an oily layer is formed, which solidifies to prismatic crystals melting at 67–68°, soluble with some dissociation in 10 parts of cold water, and giving the reactions of chloral-antipyrin.

Butylhypnal, a compound of antipyrin with butyl chloral hydrate, forms colourless needles, m. p. 70°.

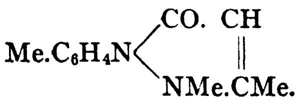
Tussol, antipyrin mandelate, $C_{11}H_{12}ON_2,C_6H_5.CHOH.CO_2H$, obtained by melting together antipyrin and mandelic acid, forms colourless crystals, m. p. 52–53°; it is sparingly soluble in water (1 in 15), easily so in alcohol. It may be recognised by its m. p., by giving a red coloration in aqueous solution (1 in 20) on adding ferric chloride, and a smell of benzaldehyde when warmed with potassium permanganate.

Migrainine (migränin) is a mixture of 90.9 parts of antipyrin with 6.6 parts of citric acid and 8.5 parts of caffeine.

To estimate antipyrin in migrainine, 1.1 grm. is dissolved in 100 c.c. of water, 20 c.c. of the solution are mixed with 20 c.c. of an alcoholic solution of mercuric chloride (2.5 grm. $HgCl_2$ to 100 c.c. of 95% alcohol) and an alcoholic solution of iodine is added which contains 1.351 grm. of iodine per 100 c.c. The iodine solution is standard-

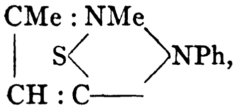
ised in the same manner against 20 c.c. of a 1% solution of pure antipyrin. 20 c.c. of the migrainine should use as much iodine as 0.2 grm. of antipyrin.

Tolypyrine is 1-*o*-Tolyl-2:3-dimethylpyrazolone,



For tests see table on following page.

Thiopyrine (Thioantipyrin)



(1-phenyl-2:3 dimethyl-2:5-thiopyrazole) is a less energetic antipyretic than antipyrin, but is said to have no injurious after-effects. It melts at 166°. It gives a transient green coloration with ferric chloride, but not with nitrous acid. The crystalline *hydrochloride* has m. p. 128°, the plantinichloride is brownish-red and melts and decomposes at 215.

Selenopyrine (selenoantipyrin), (Michaelis), forms lustrous pale yellow crystals, m. p. 168°, does not develop a coloration with ferric chloride, and only a faint green coloration with nitrous acid. The hydrochloride and sulphate do not crystallise.

Pyramidone (Dimethylaminoantipyrin, 1-Dimethylamino-2:3-dimethylpyrazolone). German Patents, 71261; 90959; 111724. M. p. 108°.

The following table, according to Hofmann (*Zeit. Unt. Nahr. Genussm.*, 1900, **6**, 419), shows differences in behaviour of antipyrin, tolypyrin, amino-antipyrin and pyramidone. (Compare, however, Monferrino, *infra*.)

	Antipyrin	Tolypyrin	Amino-antipyrin	Pyramidone
Ferric chloride.	Red-brown colour, (1 in 2000).	Red-brown colour.	Violet colour (1 in 20,000).	Blue by reflected, violet by transmitted light.
Silver nitrate.	No change.	No change.	Reddish to red-violet colour.	Colour first blue then silver then separates.
Nitrous acid.	Bright green.	Green.	Fugitive red.	Blue inclining to violet.
Nitric acid.	Warm, deep red.	Cherry red.	....	No red coloration.
Wagner's reagent.	Brown red ppt. (1 in 2000), which disappears on heating.	Brown-red ppt. (1 in 2000) which disappears on heating.	Yellowish-brown turbidity (1 in 2000), disappears on heating giving red-violet solution.	Violet colouration. Excess of the reagent gives a turbidity, which dissolves on warming.
Bromine water.	White pp.	White ppt.	Yellowish-white ppt. (1 in 2000). In 1% solution the ppt. is brilliant violet-red. Ammonia destroys the colour, sulphuric acid restores it.	Concentrated solutions give a black or gray coloration.
2% solution of blood mixed with 4 times its volume of hydrogen peroxide.	Brown colour due to meta-hæmoglobin.	Brown colour due to meta-hæmoglobin.	When H_2O_2 added a faint red colour, which on adding blood turns dark red with tendency to blue.	In very dilute solution gives violet coloration.

Distinctive Tests for Antipyrin, Pyramidone and Nevralteine[1]

Monferrino (*Boll. Chim. Farm.*, 1909, **48**, 515) states that when present together in aqueous solution the 3 compounds may be detected by the following tests:

Reagent	Antipyrin	Pyramidone
Potassium nitrite and concentrated sulphuric acid	Green coloration changing to bluish-green.	Transient amethyst-violet coloration when present in greater quantity than antipyrin.

A little of the violet liquid obtained by the addition of ferric chloride, when added to concentrated sulphuric acid, gives a green coloration if nevralteine is present.

[1] Nevralteine is sodium *p*-phenetidinemethanesulphonate.

According to Javillier, in a solution of antipyrin containing 0.7% of hydrochloric acid, silicotungstic acid produces a *white* precipitate of the composition SiO_2, $12WO_3$, $2H_2O$, $4C_{11}H_{12}ON_2$, $7H_2O$, which loses $3\frac{1}{2}H_2O$ at 120°. A visible precipitate is produced in a solution containing only 1 part of antipyrin in 10,000. Under similar conditions in solutions of pyramidone containing 0.35% of hydrochloric acid, a *yellow* amorphous precipitate, SiO_2, $12WO_3$, $2H_2O$, $3C_{13}H_{17}ON_3$, $8H_2O$ is produced which loses the whole of its water at 120°.

According to Moulin (*Ann. Chim. Anal.*, 1912, **17**, 13) pyramidone produces a blue coloration with solutions of silver or mercury nitrate. No coloration is produced with pure nitric acid but if the acid contains nitrous acid, the coloration is obtained.

Detection of Pyramidone in Urine

According to Jolles (*Zeit. anal. Chem.*, 1898, **37**, 441) when a weak solution of iodine (a 10% alcoholic solution diluted with 10 vols. of alcohol) is poured on to the surface of, but not mixed with, urine containing pyramidone, a well marked violet-red ring forms at the surface of separation and gradually changes to red-brown. The test is said to be characteristic.

Estimation of Pyramidone

Astruc and Pégurier (*Ann. Chim. Anal.*, 1905, **10**, 302) apply Lemaire's picric acid method of estimating antipyrin to the estimation of pyramidone. 0.231 grm. of the sample is dissolved in 10 c.c. of water and 40 c.c. of N/20 picric acid solution are added. After shaking during some minutes, the mixture is filtered and in 25 c.c. of the filtrate the excess of picric acid is titrated with N/10 alkali hydroxide, phenolphthalein being used as indicator. If n c.c. of alkali be used the per cent. of pyramidone $= 5(40-4n)$.

Estimation of Pyramidone in Presence of Antipyrin

Pégurier (*Ann. Chim. Anal.*, 1905, **10**, 391) utilises the fact that while antipyrin is neutral to Methyl orange, pyramidone behaves as a monoacid base with this indicator. The two bases are estimated together according to the method of Astruc and Pégurier just given. 0.231 grm. of the sample is then dissolved in 10 c.c. water, the solution exactly neutralised with N/10 acid in presence of Methyl orange,

and the antipyrin estimated by means of picric acid. For this purpose, 40 c.c. of N/20 picric acid are added, and the solution, after filtration, titrated with N/10 KOH after addition of phenolphthalein (see page 319).

Detection of Antipyrin in Pyramidone

Steensma states that it is possible to detect 0.005 mgrm. of antipyrin in 100 mgrm. of pyramidone by dissolving the latter in *p*-dimethylaminobenzaldehyde reagent (see page 318), and evaporating the solution as already described.

For tests for halogen salts in pyramidone, see Kollo, *Pharm. Post.*, 1911, 173. *Pharm. J.*, 1911, **86**, 711.

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ANALYSIS OF LEATHER

BY A. E. CAUNCE, M. Sc., F. I. C.

The study of the processes involved in tanning has been undertaken by many investigators. The many text-books on the subject should be consulted as well as articles by Herzog and Rosenburg (*Zeit. Chem. Ind. Kolloide*, 1910, **7**, 222), Procter (*J. Soc. Chem. Ind.*, 1910, **29**, 329) (British Association Report on Colloid Chemistry 1917), Gordon Parker (*J. Soc. Chem. Ind.*, 1910, **29**, 912).

The scheme of analysis of leather tanned with vegetable tannins, as originally suggested by von Schroeder, is as follows:

1. Sp. gr. found by the displacement of mercury in graduated measuring cylinder or by Simand's direct method (*Chem. Tech. Untersuchungsmethoden*, 1893, **2**, 55). Mean of 94 samples (18% H_2O) = 1.012.
2. Moisture by drying in the air at 105°.
3. Total ash, and ash of extract.
4. Fat, natural and added, and its properties.
5. Organic extractive matter (tannin and non-tannin).
6. Sugars.
7. Nitrogen by Kjeldahl's method.
8. Calcium oxide and sulphuric acid.

This method of analysis, originally devised by von Schroeder, is in use in a modified form to-day. The vegetable tanned leather is cut into thin shavings or powdered by means of a rasping machine. Under modern conditions, samples are analysed for (*a*) moisture, *b*) mineral ash, (*c*) oil, (*d*) water-soluble matter, (*e*) the mineral ash in (*d*), (*f*) hide substance is deduced from the percentage of nitrogen present.[1] The "pure leather" is estimated by difference:

$$100—[a + (b - e) + c + d] = \text{pure leather.}$$

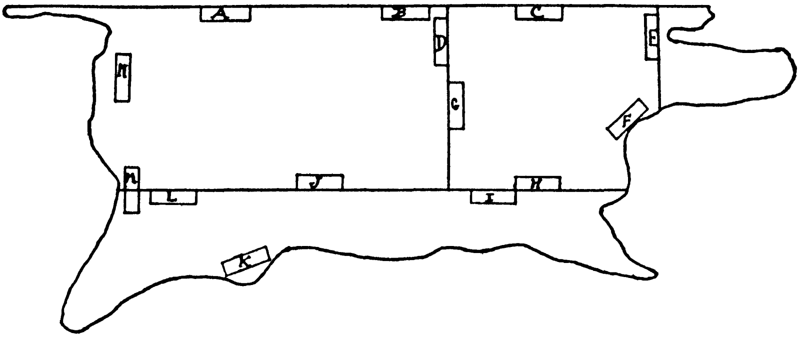
[1] Hide Substance is usually taken as containing 17.8% of nitrogen, or in the Kjeldahl estimation 1 c.c. of N/10 acid = 0.007865 grm. of hide substance.

From this figure the fixed tanning matters present may be estimated by subtracting (*f*) from the result. The ratio: fixed tanning matter/hide substance is called the "degree of tannage" and is of practical value to the tanner in determining the quality of the leather, and also to the currier or dresser of leather. It is obvious that with such a method the question of the evenness of the tanning must be taken into consideration. This can be roughly ascertained by the appearance of the section of the leather. It is found in practice that when a complete penetration is observed, and the above ratio indicates a low tannage, that this may generally be regarded as evidence of the use of some accelerating process, such as "drumming" or mechanical movement, during the tanning process, and that the tanning is correspondingly incomplete in its nature. In conjunction with the determination of such physical properties of leather as that of penetration of water, water absorption, tensile strength, etc., this ratio and other analytical details have come into general use for the estimation of its value.

A source of error in the calculation of results, due to the fact that part of the mineral ash is also extracted as soluble matter in different portions taken for these estimations, is allowed for by incinerating the water-extracted sample and deducting this from *b* as indicated: The mineral consitutents may, in this way, be estimated as water-soluble and insoluble respectively. Such an error may reduce the leather substance found by nearly 1% and correspondingly alter the "degree of tannage" returned by over 2%. It has also been pointed out that the actual figures obtained for insoluble ash may be lower than the actual insoluble mineral matter present by 0.02–0.04%, owing to certain alterations which take place on incineration (Parker and Paul, *J. Soc. Chem. Ind.*, 1910, **29**, 316), so that it is better to estimate the leather substance by subtracting the weight of insoluble ash (with possibly an addition of 0.03% for the above correction) from that of the dry leather residue after water extraction (*d*). This error would be appreciable in many leathers, owing to the bisulphite treatment and use of extracts and synthetic tans in modern processes of tanning.

No particular details are necessary in explanation of the methods used in (*a*) or (*b*). In the estimation of oil (*c*) the extraction is carried out with petroleum spirit. The water-soluble matter is estimated in the fat-extracted sample (*c*) by washing out with warm

water (45°–50°) by slow percolation. Parker and Paul advise the use of certain refinements which are set out in the accompanying illustration (Fig. 4).



	Single	Double
Belly	I–L–K	
Shoulder	C–H	G–H
Bend	A–B–J	N–D–J
Butt	A–C–J	N–E–J
Side	A–C–K	N–F–M

Fig. 4.

A water-bath, governed by a thermo-regulator, contains a number of small glass vessels or inverted Procter filter bell extractors, plugged at the end with glass wool. The flow of water, which corresponds with the specified rate of extraction employed, is so arranged that it passes through a lengthened glass tube set in the water-bath itself, so that the temperature of extraction is standardised. In practice, the temperature should never exceed 45°–50°.

It is also stated (*J. Amer. Leather Chem. Assoc.*, 1910, **5**, 426) that an error is observed in the amount of water-soluble matter when this is taken on the dried and exhausted sample as compared with that obtained when the original sample is tested.

As Procter has specially pointed out, the analysis of the ash of the leather is useful. Chromium sesquioxide, lead sulphate, tin, and antimony are the substances most likely to be found.

Vegetable tanned leathers should also be examined for adulteration with materials such as glucose, Epsom salts, barium sulphate, etc.

The microscopical examination of the leather is important and must be considered by all who are interested in the examination of leathers from the manufacturing point of view.

Estimation of Free Acid.—Many methods have been suggested for the estimation of the acidity of vegetable leathers. Usually attention is confined to the estimation of the "free" mineral acidity by an ignition method originally devised by Procter; the details of such a method are given on page 340. The result is expressed and calculated as sulphuric acid, although it is not at all certain how far other acids which may not be as harmful as sulphuric acid are estimated by the method.

PHYSICAL TESTS

The waetr-penetration test makes use of a cylinder made of copper 14 in. high and 2 in in diameter with a flange on the bottom with screw holes on which a second ring may be screwed with a 2-in. hole in the centre, like a large washer. A round piece of leather is now cut about 3 in in diameter, placed on the bottom of the cylinder and by aid of the metal washer screwed or clamped on to the bottom of the cylinder as tightly as possible. The cylinder is now filled with ordinary cold water to a height of 12 in., fastened in a retort stand, a mirror is put underneath, and the time is noted that the water takes to penetrate through the leather. The leather is always clamped in grain side upward. Tests are made generally in triplicate and the mean of the 3 is taken, and it is not sufficient to observe the time that the water takes to come through in one spot, as there may be a flaw in the leather; the leather on removal is cut with a knife to see that the water has penetrated right through. Good sole leather generally takes about 170 to 250 hours.

The Parker water absorption test is as follows: Several strips of the leather to be tested are cut 1 in. in width and about 6 in. long; they are then, by any suitable means (copper wire or otherwise), suspended in a beaker of water so that 1 in. of leather is in water. This may be marked with a pencil; the leather is then marked every ¼ in. with pencil and, at the end of 24 hours' suspension in water, the height that the water has risen by absorption is noted. The tighter and more compact the leather, the less the water absorption, but a porous, poorly tanned leather may given an absorption of 2 in., or even 3 in., in 24 hours.

The above physical tests are those adopted at the Leather Sellers' Technical College, London, and are generally recognised in the trade for their efficiency and usefulness.

The penetration of water through leather may also be tested by drawing 10 c.c. of water through a piece of leather of known thickness by means of a vacuum of known magnitude. The result is stated in terms of the quantity of water, percolating through 1 c.c. of leather in 1 minute (Thuau and Korsak, *Collegium*, 1910, 229).

The American Leather Chemists' Association has issued the following Official Methods for the sampling and the analysis of vegetable tanned leather.

Provisional Method for Sampling Leather

To sample any one of the ordinary commercial cuttings of leather, samples must be cut from each piece sampled of the size and in the locations designated below.

	Single	Double
Belly	I-L-K	......
Shoulder	C-H	G-H
Bend	A-B-J	N-D-J
Butt	A-C-J	N-E-J
Side	A-C-K	N-F-M

It is much more difficult to sample satisfactorily double than single bends, butts, and hides, and wherever possible, the sampling prescribed for single bends, butts and hide should be followed.

The location of the samples is shown only relatively because of the wide variations in the size of hides, skins etc., but the relative location shown in the diagram must be adhered to strictly.

Each sample is to be cut of rectangular shape, approximately 2.5 in. $\times$ 8 in., 0.5 in. being then removed from the uncut edge so as to leave the final sample for analysis exactly 2 in. by 8 in. The whole of each sample is to be prepared for analysis by sawing, planing or other approved method, and the leather so prepared from each sample must then be mixed with most scrupulous care to ensure a uniform mixture. Equal weights are now taken from each of the prepared samples obtained from the piece sampled, and these, when thoroughly mixed together, constitute the sample for analysis.

When samples are taken more than one piece in any one lot, all the samples from the same location are to be prepared as above and thoroughly mixed, and then equal weights taken from each of the mixtures so obtained to constitute the sample for analysis.

Sawed leather is exceedingly difficult to mix, and unless exceptional pains are taken with the mixing to insure a uniform mixture, a representative sample will not result.

OFFICIAL METHOD FOR ANALYSIS OF VEGETABLE TANNED LEATHER

1. **Preparation of Sample**—The sample of leather for analysis shall be reduced to as fine a state of division as practicable, either by cutting or grinding.

2. **Moisture.**—Dry 10 grm. of leather for 16 hours at a temperature between 95°–100°.

3. **Fats.**—Extract 5 to 10 grm. of air-dry leather in a Soxhlet apparatus until free from grease, using petroleum spirit boiling below 80°. Evaporate off the solvent and dry to approximately constant weight.

Or, if preferred, extract 30 grm. of leather as described above. In the latter case, the extracted leather, when freed from solvent, may be used for the estimation of water-soluble material.

4. **Ash.**—Incinerate 10 to 15 grm. of leather in a tared dish at a dull red heat until carbon is consumed. If it is difficult to burn off all the carbon, treat the ash with hot water, filter through an ashless filter, ignite filter and residue. Add the filtrate, evaporate to dryness and ignite.

5. **Water-soluble Material.**—Digest 30 grm. of leather in a percolator over-night, then extract with water at 50° for 3 hours. The total volume of solution to be 2 litres. Estimate total solids and non-tannins according to the Official Method for extract analysis.

6. **Glucose.**

Solutions

Copper Sulphate.—Dissolve 34.639 grm. of copper sulphate ($CuSO_4 5H_2O$) in distilled water ahd dilute to 500 c.c. Filter through asbestos.

Alkaline Tartrate Solution.—Dissolve 173 grm. of Rochelle salt and 80 grm. of sodium hydroxide in water and dilute to 500 c.c. Allow to stand two days and filter through asbestos.

Normal Lead Acetate Solution.—Prepare a saturated solution of normal lead acetate.

Gravimetric Estimation

Place 200 c.c. of leather extract of analytical strength in a 500 c.c. flask, add 25 c.c. of a saturated solution of normal lead acetate, shake frequently (5–10 minutes), and filter. (The funnels and beakers must be kept covered to prevent evaporation.) Add to the filtrate an excess of solid potassium oxalate. Mix frequently for 15 minutes and filter, returning the filtrate until clear. Pipette 150 c.c. of this filtrate into a 600 c.c. Erlenmeyer flask, add 5 c.c. of concentrated hydrochloric acid and boil under a reflux condenser for 2 hours. Cool, neutralise (place a small piece of litmus paper in the flask) with anhydrous sodium carbonate, transfer to a 200 c.c. graduated flask and make to volume. Filter through a double filter. (The filtrate must be clear.) Estimate the glucose in the solution immediately.

Place 25 c.c. of the copper solution and 25 c.c. of the alkaline tartrate solution in a 400 c.c. beaker. Add 50 c.c. of the clarified and neutralised solution above mentioned and heat to boiling in *exactly 4 minutes* and boil for 2 minutes.[1] Filter immediately without diluting, *through asbestos*,[2] wash thoroughly, with hot water, then with alcohol, and finally with ether; dry for half an hour in water oven and weigh as cuprous oxide, estimate the amount of glucose by the use of Munson and Walker's table (see Vol. I page 401) and report as percentage on leather.

Volumetric Estimation[3]

6*a*. Glucose.

Sodium Thiosulphate.—Dissolve 20 grm. of sodium thiosulphate in 1000 c.c., using boiled, cooled, distilled water. Let the solution stand 24 to 48 hours before standardising. Standardise against copper as follows: Clean a piece of pure copper foil, preferably by dipping into dilute nitric acid for a few minutes, rinsing in water, and drying with alcohol and ether. Accurately weigh 0.2 grm. of the foil into a 300 c.c. Erlenmeyer flask, dissolve in 5 c.c. of dilute nitric

[1] The rate of heating of the Bunsen burner used should be regulated before sugar estimations are started. This is best done by adjusting the burner so as to bring 25 c.c. copper soln. + 25 c.c. alk. tartrate soln. + 50 c.c. water in a 400 c.c. beaker to 100° in exactly 4 minutes.

[2] The finely divided, long-fibred asbestos to be used in the glucose estimation should be digested with nitric acid, washed, then digested with sodium hydroxide and washed. When Gooch filters are prepared, they should be washed with boiling Fehling's solution, then with nitric acid. The mats thus prepared can be used for a long time.

[3] This method has recently been adopted as the standard official method of the American Association of Leather Chemists (*J. Amer. Leather Chem. Assoc.*, 1925, **10**, 448).

acid (1 to 1), add 45 c.c. of water and a pinch of powdered pure talc, boil for about twenty minutes remove and cool. From here proceed exactly as described later in the method starting with "Add ammonium hydroxide, carefully . . ." From the weight of copper foil used, calculate the number of milligrams of cuprous oxide equivalent to one c.c. of the thiosulphate solution. Keep the thiosulphate solution in a dark bottle.

Estimation

Place 200 c.c. of leather extract of analytical strength in a 500 c.c. flask, add from a pipette 25 c.c. of saturated normal lead acetate, shake frequently during five to ten minutes, and filter. (Keep funnels covered during all filtrations.) Add to the filtrate sufficient solid potassium oxalate to completely precipitate the excess of lead. Mix frequently during fifteen minutes and filter, returning the filtrate until clear.

Pipette 150 c.c. of this filtrate into a 600 c.c. Erlenmeyer flask, add 5 c.c. of concentrated hydrochloric acid and boil under a reflux condenser for two hours. Cool to about 15°, and neutralise to Methyl Red with saturated sodium hydroxide solution. (The saturated sodium hydroxide solution should be run from a burette so that its addition can be carefully controlled.) Transfer to a 200 c.c. graduated flask and make to volume. Pipette 50 c.c. of this solution (unfiltered) into a mixture of 25 c.c. of the copper sulphate solution and 25 c. c. of the alkaline tartrate solution contained in a low form 400 c.c. beaker of as near to 7 to 8 cm. inside diameter and 9 to 10 cm. depth as possible. Heat to 100° in exactly four minutes, and continue heating for exactly two minutes, using thermometers to control this operation. Filter immediately, without diluting, through asbestos, and wash thoroughly with hot water.

Cover the Gooch crucible with a watch glass, and dissolve the cuprous oxide with about 10 c.c. of warm dilute nitric acid. (1 to 1) poured under the watch glass with a pipette. Collect the solution in a 300 c.c. Erlenmeyer flask, using a bell jar and suction. Wash the watch glass and Gooch crucible entirely free of copper, filling the crucible three or four times with hot water. Rinse the outside of the crucible and also the holder. To the copper nitrate solution add a pinch of powdered pure talc, boil on a hot plate for about twenty

minutes, or until the volume is about 50 c.c., remove and cool. Add ammonium hydroxide carefully until copper hydroxide begins to precipitate or until a hazy pale blue colour appears, but not sufficient to develop a deep blue colour. If too much ammonium hydroxide is accidentally added, the solution must be re-acidified with dilute nitric acid which has been boiled with talc, and the neutralisation repeated. After neutralisation add 4 to 5 c.c. of 80% acetic acid to dissolve the copper hydroxide, then add 10 c.c. of a 30% potassium iodide solution (3 grm. of potassium iodide must be used in each titration, even though little copper is present) and titrate with the standardised sodium thiosulphate solution.

When most of the brown colour has disappeared, add a little starch solution and complete the titration. At the completion of the titration the volume of the solution must not exceed 150 c.c. From the titration estimate the mg. of cuprous oxide, and calculate the amount of glucose from Munson & Walker's table and record as percentage of the leather.

7. **Provisional Method for Detemrination of Epsom Salts in Vegetable-Tanned Leather.**—Ash 5 or 10 grm. of leather; carefully moisten ash with water; add 15 c.c. of concentrated hydrochloric acid; wash into a beaker; dilute to 50–75 c.c.; add 2–3 drops of concentrated nitric acid; gently boil for a few minutes or heat on a steam-bath for 15 minutes. Without filtering off insoluble matter, add ammonium hydroxide (approximately 1 to 1) slowly, with constant stirring, until nearly neutral but still slightly acid, then add dilute ammonium hydroxide (about 3 or 4 to 1) and precipitate with a very slight excess of it. (If the precipitate does not have the characteristic reddish brown colour of ferric hydroxide and there is known to be sufficient ammonium chloride present to hold in solution all magnesium, redissolve in hydrochloric acid without filtering, add a few drops of pure ferric chloride solution and precipitate with ammonium hydroxide.) Boil for a few minutes; filter and wash the precipitate thoroughly with hot water. If necessary, evaporate the filtrate to 175–200 c.c. and make ammoniacal (about 1 c.c. ammonium hydroxide); boil gently, and add slowly with constant stirring 10 c.c. of a saturated ammonium oxalate solution; cover and leave for 2 hours or longer on a steam-bath or in a warm place. Quantitatively wash solution and precipi-

tate into a 250 c.c. volumetric flask; cool to 20–25°; fill to the mark with distilled water, and mix thoroughly. Filter through quantitative paper, making sure that filtrate is absolutely clear. Pipette an aliquot part equivalent to 2 grm. of original leather, and dilute to about 150 c.c. Make slightly acid with hydrochloric acid (Methyl Orange), cool if necessary, add a slight excess of clear saturated ammonium hydrogen phosphate solution (5 c.c. generally sufficient); while stirring vigorously, add a few drops of ammonium hydroxide until precipitation just starts or until faintly ammoniacal; leave for 15 minutes; add, with stirring, 5 c.c. concentrated ammonium hydroxide; cover and leave overnight at room temperature and proceed by the gravimetric or volumetric method.

Gravimetric.—Filter through a well-prepared Gooch crucible; wash the precipitate free from chlorides with 1 part concentrated ammonium hydroxide (sp. gr. 0.90) to 9 parts water; finally just moisten the precipitate with a few drops of a solution of approximately 50% ammonium nitrate in 1 to 9 ammonia water; dry; ignite gently at first, then cover the crucible and ignite intensely for 20–30 minute intervals until constant in weight; weigh as $Mg_2P_2O_7$, multiply by factor to convert to $MgSO_4 7H_2O$, and express as % on 2 grm. of leather.

Volumetric.—Filter clear, through close quantitative paper; wash the precipitate free from chlorides with 1 part concentrated ammonium hydroxide (sp. gr. 0.90) to 9 parts of water; remove excess of ammonia wash water, either by washing 3 or 4 times with neutral 60% by volume methyl alcohol solution; or by spreading out the filter paper with its precipitate on coarse absorbent filter paper for a couple of minutes and then on a watch glass, and dry for 1 hour at 50°, (if 60° is exceeded, estimation must be discarded); or by air-drying the opened-out filter with its precipitate overnight at room temperature. After removal of ammonia transfer paper with its precipitate to a beaker or flask; moisten with water; thoroughly disintegrate the paper; add an accurately measured excess of standardised N/10 sulphuric acid with 2 or 3 drops of Methyl Orange (0.1% alcoholic solution). Dilute to about 100 c.c. and estimate excess of acid by titrating with N/10 sodium hydroxide to a clear yellow without any suggestion of pink. 1 c.c. of N/10 sulphuric acid is equivalent to 0.0123 grm. $MgSO_4 7H_2O$. Calculate to grm. $MgSO_4.7H_2O$ and expresses % on 2 grm. of leather.

8. Nitrogen.—Gunning modification of the Kjeldahl Method, A. O. A. C. Bulletin, No. 107 (1904). (See Vol. I.)

Reagents

Standard Acid Solutions.—Hydrochloric or sulphuric acid the absolute strength of which has been accurately ascertained. For ordinary work N/2 acid is recommended. For work with very small amounts of nitrogen, N/10 is recommended. In titrating mineral acid against hydroxide solution use Cochineal as indicator.

Standard Alkali Solution.—The strength of this solution relative to the acid must be accurately known; N/10 solution is recommended.

Sulphuric Acid.—The sulphuric acid used should have a sp. gr. 1.84 and be free from nitrates and also from ammonium sulphate.

Sodium Hydroxide Solution.—A saturated solution of sodium hydroxide free from nitrates.

Potassium Sulphate.—This reagent should be pulverised before using.

Indicator.—A solution of Cochineal is prepared by digesting and frequently agitating 3 grm. of pulverised Cochineal in a mixture of 50 c.c. of strong alcohol and 200 c.c. of distilled water for a day or two at ordinary temperature; the filtered solution is employed as indicator.

Estimation

Place 1.5 grm. of a sawn preparation, or its equivalent in degree of subdivision, in a digestion flask. Add 10 grm. of powdered potassium sulphate and from 15 to 25 c.c. (ordinarily about 20 c.c.) of concentrated sulphuric acid. Place the flask in an inclined position and heat below the boiling-point of the acid from 5 to 15 minutes or until frothing has ceased (a small piece of paraffin wax may be added to prevent extreme foaming).

Then raise the temperature and boil briskly until the liquid has become quite clear and nearly colourless (the digestion should take from 4 to 5 hours).

After cooling, dilute with about 200 c.c. of water. Next add 50 c.c. of sodium hydroxide solution, or sufficient to make the reaction strongly alkaline, pouring it down the side of the flask, so that it does not mix at once with the acid solution. Connect the flask

with the condenser, mix the contents by shaking, and distil until all ammonia has passed over into the standard acid. The first 150 c.c. will generally contain all the ammonia. The operation usually requires from 40 minutes to 1½ hours. The distillate is then titrated with standard alkali.

Previous to use, the reagents should be tested by a blank experiment with sugar, which will partially reduce any nitrates present that otherwise might escape notice.

9. **Provisional Method for the Estimation of Mineral Acidity of Vegetable Tanned Leathers.**—Weigh 2 grm. of the finely divided, thoroughly mixed sample into a platinum or rhotanium dish. Add 40 c.c. of standard N/10 sodium carbonate solution, mix thoroughly, and evaporate to complete dryness on steam-bath. Ignite the residue at a dull red heat, preferably in a muffle furnace, until the carbon has been nearly burned off, giving a residue showing not over one-third carbonaceous matter and two-thirds ash. Maintain the temperature at a dull red heat. If too high a temperature is reached sodium carbonate will be lost and a high value for acid will be obtained. Allow the residue to cool, and moisten carefully with hot water, adding about 25 c.c. and breaking up the residue with a glass rod. Filter into a 300 c.c. Erlenmeyer flask through an ashless filter paper, and wash four or five times with hot water. Return the filter paper and residue to its dish, dry and ignite at dull red heat until all the carbon has been burned off. Cool and add to the residue from a burette a quantity of standard N/10 sulphuric acid exactly equivalent to the standard sodium carbonate originally added. Cover the dish and place on a steam bath for 30 minutes. If a clear solution is obtained, transfer it quantitatively to the flask containing the first filtrate. If there is a residue which is sufficient to interfere with the end point of the titration, filter the solution through a quantitative filter paper into the flask containing the first filtrate washing the paper thoroughly with hot water until free from acid. Cool the solution and titrate to a clear yellow colour with N/10 sodium hydroxide or N/10 sodium carbonate and 2 or 3 drops of Methyl Orange indicator. Express the result as % sulphuric acid.

With each set of estimations it is desirable to run at least one blank estimation, using the standard solutions. If the blank is over 0.3 c.c. it is best to repeat the estimation.

Tannins.—The detection of the nature of the tannins that have been used in the process of manufacture, may be carried out by stripping the tannin from the leather by the action of a dilute solution of a mild alkali such as borax or sodium carbonate and subsequent acidification. Kohnstein (*Allgem. Gerber. Zeit.*, 1912, **14**, 5) uses the water extract for this purpose. Procter (Leather Industries Laboratory Book (1919)) gives very full details for the identification of the vegetable tannins. The presence and detection of sulphite-cellulose in leather has been dealt with by W. Moeller (*Collegium*, 1914, **531**, 489), while Grasser (Synthetic Tannins, Grasser and Enna, 1922) has made an extensive study of the properties and methods of testing for the synthetic tans, generally of coal-tar origin, which have recently found such an extended use in leather manufacture.

Seel, Hils and Reihling (*Z. angew. Chem.*, 1919, **32**, 4) give analyses of German leathers tanned with synthetic tannins.

Analysis of East Indian Tanned Hides.—M. C. Lamb (*Tanners' Year Book*, 1913, 165) has given many analyses indicating that on the whole these tanned hides are fairly pure. In a typical example the following results were obtained:

Leather fibre	68.86%
Oil and fatty matter	8.43%
Water-soluble matter	8.6 %
Moisture	13.7 %
Mineral matter	1.12%

Procter also estimates the insoluble residue after extraction, and estimates the nitrogen in this to ascertain the actual hide substance present.

Methods of analysis used in other countries than America are based on the same general procedure, but differ from it in various manipulative details, notably in the estimation of water-soluble matter and moisture.

The British Government has accepted a method of sampling and analysing sole leather (*J. Soc. Leather Trade Chem.*, 1918, **2**, 51) which is the method generally used in Great Britain for vegetable leathers. A method of reporting results is also given.

The regulations of the Swedish Government (*Ibid.*, 1921, **5**, 198) prohibit the adulteration or undue weighting of imported leather with glucose, mineral salts, excess tannin, etc., and details of sam-

ANALYSIS OF AMERICAN LEATHERS

Place sample was obtained	Time sample was obtained	Description of sample	Tannage claimed	Moisture, per cent.	Ash, per cent.	Petroleum spirit extract, per cent.	Water-soluble material: Tannins, per cent.	Water-soluble material: Non-tannins, per cent.	Water-soluble material: Total soluble, per cent.	Epsom salts, per cent.	Glucose, per cent.	Combined tannins, per cent.	Hide substance, per cent.	Ratio of combined tannins to hide substance
Boston, Mass.	1906	Sole, supposed to be weighted	Oak	7.5	0.95	1.5	12.7	14.0	26.7	......	5.4	26.1	37.9	0.69
Do.	1907	No. 1, Texas oak sole	Do.	6.9	0.8	4.9	17.0	6.8	23.8	......	1.5	24.7	39.4	0.63
Do.	Do.	No. 2, Texas oak sole	Do.	6.5	2.1	1.7	10.2	18.9	29.1	3.9	5.7	30.9	31.5	0.98
Do.	Do.	No. 3, Hemlock sole	Hemlock	7.3	1.9	2.2	14.8	11.9	26.7	1.5	7.2	25.8	37.7	0.68
Do.	Do.	No. 4, Oak sole	Oak	6.9	1.2	4.0	15.1	6.4	21.5	0.5	2.6	29.2	38.1	0.77
Do.	Do.	No. 5, English pure bark	....	7.3	2.3	1.3	14.2	11.3	25.5	0.7	0.7	27.0	38.6	0.70
Do.	Do.	No. 6, Hemlock sole	Hemlock	7.7	1.0	1.3	17.7	11.5	29.2	1.2	4.6	24.1	37.4	0.64
Baltimore, Md.	Do.	Oak sole	Oak	6.8	2.6	1.0	14.9	18.4	33.3	4.7	12.0	21.4	37.2	0.58
Do.	Do.	Union sole	Union	8.3	2.7	2.5	13.2	14.8	28.0	6.4	6.1	27.5	33.4	0.82
Do.	Do.	Texas oak sole	Oak	8.3	1.7	1.5	13.7	13.1	26.8	4.0	5.3	28.5	34.6	0.82
Do.	Do.	Hemlock sole	Hemlock	6.3	1.8	0.4	7.5	19.2	26.7	4.6	10.0	29.5	36.8	0.80
Do.	Do.	Texas scoured oak	Oak	7.3	0.7	2.0	14.8	7.1	21.9	0.4	2.3	28.0	40.5	0.69
Boston, Mass.	Do.	Drum tanned	....	7.6	0.9	0.7	13.4	2.9	16.3	1.2	0.0	26.1	49.0	0.53
Do.	Do.	Do.	....	7.9	0.9	1.0	10.2	3.2	13.4	0.4	0.2	27.5	49.9	0.55
Unknown	Do.	No. 1 sole, No. 2 bend	Union	8.8	1.9	3.2	14.4	16.1	30.5	4.4	9.2	23.8	33.4	0.71
Alexandria, Va.	Do.	Scoured oak sole, No. 1 bend	Oak	4.4	0.3	4.7	15.6	4.6	20.2	......	1.2	33.0	37.4	0.88
Do.	Do.	Do.	Do.	3.6	0.2	3.3	14.9	5.1	20.0	......	1.8	32.5	40.3	0.81
Do.	Do.	Scoured oak, extract tanned (in wheel after vat)	Do.	3.8	0.3	3.5	14.2	5.0	19.2	......	1.5	33.3	39.9	0.83
Do.	Do.	Vat-tanned belting, No. 1 butt	....	4.9	0.2	5.3	11.5	5.5	17.0	......	1.3	34.5	38.0	0.91
Do.	Do.	No. 2 side	Hemlock	5.8	0.9	3.3	14.8	12.1	26.9	1.4	6.2	28.0	35.7	0.78
Do.	Do.	Oak	Union	5.9	1.2	4.5	14.2	9.7	23.9	2.4	2.7	30.8	34.6	0.89
Washington, D. C.	Do.	Scoured oak	Oak	6.3	1.0	5.5	13.6	10.8	24.4	3.0	2.5	27.7	35.8	0.77
Do.	Do.	Do.	Do.	7.0	0.6	3.7	13.2	6.8	20.0	......	1.5	32.4	36.6	0.89
Do.	Do.	Do.	Do.	6.3	0.5	4.4	13.5	7.7	21.2	......	2.0	28.0	39.8	0.70
Do.	Do.	Do.	Do.	5.5	1.0	1.9	12.6	17.9	30.5	2.1	7.2	26.8	35.0	0.77
Do.	Do.	Hard-rolled scoured oak	Do.	8.0	1.9	2.9	15.4	15.0	30.4	6.1	5.5	25.9	32.5	0.80
Do.	Do.	Do.	Do.	8.2	1.4	2.3	14.9	12.9	27.8	4.4	4.4	27.5	33.9	0.81
Do.	Do.	Scoured oak	Do.	6.9	1.5	2.5	10.1	12.5	22.6	1.0	6.1	27.7	40.0	0.69
Do.	Do.	Do.	Do.	7.7	1.5	3.1	9.3	10.7	20.0	0.5	5.1	26.4	42.5	0.62
Do.	Do.	Do.	Do.	8.9	2.4	1.2	12.5	14.9	27.4	2.8	4.0	30.0	32.2	0.93
Do.	Do.	Do.	Do.	8.2	1.4	1.5	13.4	11.6	25.0	4.1	2.4	29.5	35.5	0.83
Do.	Do.	Texas scoured oak	Do.	8.0	0.5	4.1	15.3	6.5	21.8	0.6	0.6	25.1	40.7	0.62
Do.	Do.	Do.	Do.	7.7	0.4	3.6	15.3	2.4	17.7	0.4	0.8	29.1	41.6	0.70
Do.	Do.	Scoured	Do.	7.6	1.0	4.4	9.4	10.9	20.3	3.2	2.8	26.7	40.7	0.66

Do.	Do.	Do.	Do.	8.9	0.9	3.6	11.6	8.5	20.1	2.3	2.1	23.3	43.8	0.53
Do.	Do.	Scoured oak sole	Do.	8.1	0.5	1.9	14.7	6.7	21.4	1.0	0.5	30.9	37.4	0.82
Do.	Do.	Do.	Do.	7.7	0.4	1.5	10.9	7.6	18.5	1.2	1.2	31.1	40.9	0.76
Baltimore, Md.	1912	Scoured oak	Do.	7.2	1.4	1.6	14.8	19.5	34.3	4.9	9.6	23.6	33.0	0.72
Do.	Do.	Do.	Do.	7.4	1.0	4.1	13.1	14.8	27.9	2.1	6.0	25.3	35.0	0.72
Do.	Do.	Texas scoured oak	Do.	8.2	0.6	3.4	11.9	7.5	19.4	0.3	1.6	32.3	36.4	0.88
Do.	Do.	Do.	Do.	8.9	0.4	2.0	13.6	5.9	19.5	......	1.4	30.0	39.3	0.76
Do.	Do.	Scoured oak	Do.	8.8	1.5	1.0	13.6	18.7	32.3	4.5	3.5	25.2	32.4	0.78
Do.	Do.	Hemlock sole	Hemlock	8.3	2.5	1.3	9.3	22.1	31.4	5.7	12.4	25.7	33.0	0.78
Do.	Do.	Do.	Do.	7.6	1.6	1.6	9.7	18.1	27.8	6.4	6.4	24.2	38.5	0.63
Do.	Do.	Union sole	Union	8.7	1.5	2.5	14.6	13.4	28.0	4.1	5.5	27.4	33.1	0.83
Do.	Do.	Do.	Do.	7.4	2.0	3.7	13.5	11.0	24.5	3.8	5.4	28.5	35.6	0.80
Do.	Do.	Hemlock sole	Hemlock	8.4	1.2	1.2	12.0	17.5	29.5	2.8	10.5	28.9	31.7	0.91
Do.	Do.	Do.	Do.	7.7	0.9	1.1	14.1	14.9	29.0	2.0	8.2	27.3	34.6	0.78
Do.	Do.	Scoured oak	Oak	8.2	0.3	1.3	13.7	6.1	19.8	......	1.5	33.1	37.3	0.89
Do.	Do.	Do.	Do.	8.2	0.4	1.1	14.0	5.9	19.9	......	1.2	31.3	39.2	0.80
Do.	Do.	Do.	Do.	12.3	0.5	0.9	10.9	7.1	18.0	......	1.2	28.9	39.6	0.73
Do.	Do.	Do.	Do.	7.9	0.5	0.7	10.5	7.3	17.8	......	1.5	32.4	40.9	0.79
Do.	Do.	Do.	Do.	8.4	1.2	1.1	13.2	14.5	27.7	1.9	5.9	27.0	35.5	0.76
Do.	Do.	Do.	Do.	8.6	0.9	2.3	12.0	12.6	24.6	2.5	5.6	27.0	37.2	0.73
Do.	Do.	Vat (no extract) scoured oak	Do.	7.5	0.3	2.9	11.5	6.1	17.6	......	1.8	29.5	42.2	0.69
Do.	......	Do.	Do.	7.6	0.3	2.6	12.9	4.7	17.6	......	0.5	29.0	42.9	0.68
Alexandria, Va.	Do.	Vat, chestnut oak extract, scoured oak	Do.	7.9	0.3	1.9	12.6	4.6	17.2	......	0.4	34.2	38.5	0.89
Do.	Do.	Vat, chestnut oak extract, and quebracho	Do.	7.3	0.4	2.4	14.4	4.7	19.1	0.3	0.5	34.4	36.5	0.94
Do.	Do.	Do.	Do.	7.9	0.4	3.7	12.4	5.5	17.9	......	0.4	33.3	36.9	0.90
Do.	Do.	Do.	Do.	8.2	0.2	1.5	14.4	6.3	20.7	......	0.6	34.4	34.9	0.99
Do.	Do.	Do.	Do.	8.4	0.2	1.8	13.8	5.8	19.6	......	0.5	34.8	35.1	0.99
St. Louis, Mo.	1909	No. 1, Texas oak	Do.	5.8	0.7	3.6	17.3	9.8	27.1	......	2.7	25.4	37.8	0.67
Do.	Do.	No. 2, union	Union	5.1	2.4	5.6	14.7	18.9	33.6	7.5	6.5	25.1	30.3	0.83
Do.	Do.	No. 3, extract tanned, oak	Oak	4.1	1.9	5.6	12.9	13.1	26.0	3.5	....	27.1	36.9	0.73
Do.	Do.	No. 4, oak	Do.	4.1	1.3	4.1	14.5	12.7	27.2	3.1	3.6	26.8	37.5	0.71
Do.	Do.	No. 5, extract tanned, union	Union	4.2	1.6	2.5	16.2	10.2	26.4	3.9	3.4	28.3	38.3	0.74
Do.	Do.	No. 6, extract tanned	Do.	3.4	1.0	4.0	14.2	11.4	25.6	2.9	4.3	29.3	37.4	0.78
Do.	Do.	No. 7, weighted, hemlock	Hemlock	3.6	0.9	1.8	11.7	12.9	24.6	2.1	....	27.1	42.6	0.64
Do.	Do.	No. 8, hemlock	Do.	4.7	1.2	1.9	11.6	17.4	28.7	2.1	5.9	29.7	34.7	0.86
Do.	Do.	No. 9, extract, union	Union	4.2	1.1	2.0	14.2	11.3	25.5	2.7	3.7	28.7	39.3	0.73
Do.	Do.	No. 10, adulterated, South American dry hide	....	4.7	2.7	2.9	15.5	21.2	36.7	3.8	2.8	25.8	29.6	0.87
Do.	Do.	No. 11, belly, oak	Oak	5.4	0.5	3.8	11.4	13.0	24.4	......	3.7	25.7	40.4	0.64
Do.	Do.	No. 12, extract, hemlock	Hemlock	5.6	0.4	1.4	15.8	7.6	23.4	......	....	26.2	43.1	0.60
Do.	Do.	No. 13, so-called oak	Oak	6.3	1.5	3.5	9.2	12.2	24.4	4.7	3.6	27.3	38.2	0.71
Do.	Do.	No. 14, oak	Do.	4.3	0.7	3.4	6.1	10.6	16.7	......	5.2	29.1	46.2	0.63
Do.	Do.	No. 15, oak back	Do.	4.8	0.8	4.3	13.7	10.3	24.0	......	5.6	26.7	39.9	0.67
Do.	Do.	No. 16, weighted, hemlock	Hemlock	5.7	1.0	2.3	14.9	20.2	35.1	3.4	10.7	24.4	32.2	0.76
Do.	Do.	No. 17, hemlock	Do.	4.0	0.9	2.2	14.3	8.6	22.9	1.3	3.1	27.6	42.1	0.66

ANALYSIS OF AMERICAN LEATHERS.—(*Continued*)

Place sample was obtained	Time sample was obtained	Description of sample	Tannage claimed	Moisture, per cent.	Ash, per cent.	Petroleum spirit extract, per cent.	Water-soluble material: Tannins, per cent.	Water-soluble material: Non-tannins, per cent.	Water-soluble material: Total soluble, per cent.	Epsom salts, per cent.	Glucose, per cent.[3]	Combined tannins, per cent.	Hide substance, per cent.	Ratio of combined tannins to hide substance
Do.	Do.	No. 18, South American dry hide, weighted	Do.	4.3	1.0	2.9	13.7	17.3	31.0	4.3	5.6	30.0	31.5	0.95
Do.	Do.	No. 19, best	Chrome[1]	4.0	6.7	32.1	0.2	2.5	2.7	......	1.4	......	47.0	......
Do.	Do.	No. 20, best	Chrome[2]	2.9	6.0	34.6	0.2	2.9	3.1	......	1.4	......	54.9	......
Portsmouth, Ohio	Do.	No. 1	Union	5.9	1.5	3.1	13.4	14.5	27.9	4.9	5.5	26.4	36.4	0.73
Do.	Do.	No. 2, oak	Oak	5.7	0.6	2.1	10.4	13.5	23.9	......	8.6	25.6	42.4	0.60
Do.	Do.	No. 3	Union	5.9	0.8	2.5	13.5	9.7	23.2	2.6	2.8	30.1	38.0	0.79
Do.	Do.	No. 4	Oak	5.2	0.3	3.5	11.7	7.8	19.5	......	1.2	30.7	40.8	0.75
Do.	Do.	No. 5	Do.	5.8	0.3	1.4	12.0	7.6	19.6	......	1.5	31.0	41.9	0.74
Do.	Do.	No. 6	Do.	5.6	0.4	0.9	8.9	19.6	28.5	0.2	8.0	24.6	40.1	0.60
Do.	Do.	No. 7	Do.	6.4	0.3	1.3	12.2	7.0	19.2	......	0.9	29.4	43.4	0.68
Do.	Do.	No. 8	Union	4.8	0.9	2.5	12.1	9.8	21.9	0.2	3.8	28.0	42.5	0.66
Unknown	Do.	Sole, lot No. 110, iron, not treated	Do.	5.9	0.4	4.7	9.8	3.7	13.5	......	1.0	28.1	47.5	0.59
Do.	Do.	Sole, lot No. 210, iron, treated about 6%	Do.	6.2	2.3	2.0	10.0	13.5	23.5	0.2	9.2	26.7	41.3	0.65
Do.	Do.	No. 12	Do.	5.6	0.8	1.2	12.0	12.1	24.6	......	0.8	27.3	41.0	0.67
Do.	Do.	Dark color	Do.	5.3	0.2	4.3	12.2	5.8	18.0	......	1.4	36.4	35.7	1.01
Do.	Do.	Do.	Do.	5.4	0.3	2.9	10.7	5.9	16.6	......	1.0	40.3	34.5	1.16
Do.	Do.	Green yellow cast	Oak	4.9	0.3	2.5	14.1	4.3	18.4	......	0.8	31.2	42.7	0.73
Do.	Do.	Green yellow, redder than above	Do.	5.6	0.3	1.4	14.5	4.4	18.9	......	0.9	31.8	42.0	0.75
Do.	Do.	Hemlock back	Hemlock	5.6	0.8	3.2	14.4	13.9	28.3	......	9.7	32.4	30.2	1.07
Washington, D. C.	1910	Oak	Do.	9.3	1.2	1.1	17.8	7.7	25.5	1.2	2.5	29.3	34.5	0.85
Alexandria, Va.	1911	Scoured oak sole	Oak	7.2	0.2	1.9	13.9	5.3	19.2	......	0.7	34.0	37.5	0.90
Washington, D. C.	Do.	Flexible oak bend	Do.	8.2	0.4	3.9	11.7	18.5	30.2	......	10.2	23.5	33.9	0.69
Do.	Do.	Oak sole	Do.	8.9	0.2	4.0	12.9	5.3	18.2	......	0.7	26.9	41.8	0.64
Portsmouth, Ohio	1912	......	Union	6.3	0.7	2.6	13.0	8.5	21.5	1.9	2.3	27.7	41.6	0.66
Do.	Do.	......	Do.	5.9	1.0	1.1	11.2	10.1	21.3	3.8	4.2	29.2	42.2	0.69
Do.	Do.	......	Do.	6.8	1.5	2.9	12.5	11.8	24.3	4.6	4.6	27.8	37.9	0.73
Do.	Do.	......	Hemlock	5.6	1.9	1.6	13.3	14.4	27.4	2.5	6.9	28.4	36.7	0.77
Do.	Do.	......	Oak	5.9	0.7	2.5	14.8	7.7	22.5	1.5	1.7	28.7	40.1	0.71
Average	......	......	......	6.6	1.1	3.4	12.8	10.5	23.3	2.7	3.8	28.5	38.2	0.75
Average in weighted samples			......			......	......	......	......	3.0	5.5	......	......	......

[1] Sample No. 9713: 5.02% chromium trioxide in original sample.
[2] Sample No. 9714: 1.32% chromium trioxide in original sample; 0.30% chromium trioxide in water-scluble substances.
[3] Procter allows anything over 2% of gluclose as adulteration.

pling and analysis are given in the Proclamations. In France new standards of analysis (*Le Cuir*, 1923, 118 and 366) have recently been adopted. In an extended inquiry by Veitch and Rogers (*J. A. L. C. A.*, 1912, **7**, 127) it was observed that no less than 63% of the leathers examined (American), were loaded with glucose or Epsom salts. The results are given in detail: (See pages 342 to 344). More recently, Veitch, Frey and Clark (*U. S. A. Dept. of Agric. Bull.*, 1168) have carried out an exhaustive investigation to correlate the products of various tannages, their details of analysis, and wearing qualities under army conditions.

The details of vegetable leather analysis have been the subject of a large amount of investigation. Jablonski (*Collegium*, 1922, 379) reports the recommendations of the German Commission. Chambard (*J. S. L. T. C.*, 1924, **8**, 87) gives the details of the French methods, and claims them to give results as reproducible as the American method. At the recent Conference of the Society of Leather Trades' Chemists at Barcelona an International Committee was appointed to draw up a scheme of analysis that would embody the results of all the recent work on the subject, and cooperate with the American and Swedish chemists to produce a method of universal acceptance.

The variations of the analysis figures with the position of the sample taken are given in detail by F. H. Small (*J. Amer. L. C. Assoc.*, 1921, **16**, 394). Veitch and Frey (*J. Amer. L. C. Assoc.*, 1918, **13**, 232) point out that in the preparation of the sample, heating during grinding affects the moisture and water-soluble figures. Balderston (*J. Amer. L. C. Assoc.*, 1923, **18**, 154) confirms this, recommends slicing and describes a suitable machine for the purpose. Blockey (*J. S. L. T. C.*, 1922, **6**, 384) advises planing.

The use of other fat solvents than the generally used petroleum spirit has been investigated (*J. S. L. T. C.*, 1920, **5**, 7 and 300), and has also received attention from Schultz (*J. S. L. T. C.*, 1922, **6**, 389) and Balderston (*J. Amer. L. C. Assoc.*, 1923, **18**, 475). Hey (*J. S. L. T. C.*, 1922, **6**, 385) has shown the influence of moisture upon the estimation of fat with various solvents.

Jalade (*Le Cuir.*, 1919, **8**, 394) Chater and Woodroffe, (*J. S. L. T. C.*, 1922, **6**, 247) and Schultz (*J. Amer. L. C. Assoc.*, 1923, **18**, 254) review the water-soluble estimation.

Kohn and Crede (*J. Amer. L. C. Assoc.*, 1923, **18**, 189) describe a new method for the measurement of the acidity of leather that is of interest.

Chrome Leather Analysis.—In the analysis of chrome leather, which contains no tannin, certain modifications are necessary. The moisture, ash, and chromium contents are estimated on one portion of the sample. A separate sample is extracted with petroleum spirit and, after drying, it is extracted with water in a manner similar to that used for vegetable leather. After again drying, an estimation of the basicity of the chromium fixed on the leather fibre is made by estimating the chromium in one part of the leather so extracted, and the sulphuric acid (SO_4) in another portion of equal weight. Nitrogen or hide substance is estimated in the usual way. For the estimation of the chromium the American Leather Chemists Association have issued the following method.

Provisional Method for the Analysis of Chrome Leather
Chrome Estimation

(*a*) Ash 3 grm. of leather. Mix the ash well with 4 grm. of a mixture of equal parts of sodium carbonate, potassium carbonate and powdered borax glass and fuse for 30 minutes. Dissolve the cooled fused mass in hot water with enough hydrochloric acid to make the solution acid. Filter. If there is any residue on the filter, ash it and treat the ash with 1 grm. of the fusion mixture in the same way as the original ash, adding the solution to the first. Make up to 500 c.c. To 100 c.c. of this solution in an Erlenmeyer flask, add 5 c.c. of hydrochloric acid, then add 10 c.c. of a 10% solution of potassium iodide. After 1 minute run in from a burette N/10 sodium thiosulphate until the iodine colour has nearly disappeared; then add a few c.c. of starch solution (1 grm. per 1000 c.c.) and titrate to the disappearance of the blue colour. One c.c. of N/10 thiosulphate solution is equivalent to 0.002533 grm. Cr_2O_3.

(*b*) If it is not desired to estimate iron or aluminium, the ash of 3 grm. of the leather may be transferred to an iron crucible, mixed with 3 grm. of sodium peroxide and fused 10 minutes. Place cooled crucible in 300 c.c. water in a basin, and boil 20 minutes. Wash into a 500 c.c. flask, cool and make up to the mark. Filter through a dry filter. Place 100 c.c. of filtrate in an Erlenmeyer flask, neutralise with hydrochloric acid add 5 c.c. excess and proceed as in (*a*).

Recently the chrome leather analysis committee of the American Society have published (*J. Amer. Leather Chem. Assoc.*, 1924, **19**, 191) a recommended procedure for the sampling and analysis of chrome-tanned leather which includes the estimations of moisture, fat, ash, chromium, barium sulphate, iron, aluminum, and hide substance. They recommend the adoption of Schorlemmer's method (*Collegium*, 1920, 536) for the expression of basicity.

Basicity figure is:

$$\frac{\text{Amount of } Cr_2O_3 \text{ combined with hydroxyl} \times 100}{\text{Total } Cr_2O_3}$$

In the Report of the International Commission on Chrome Leather Analysis, Innes (*J. S. L. T. C.*, 1923, **7**, 431) summarises the present position of chrome leather analysis and gives recommended details for the estimation of moisture, ash, chromium and the expression of the basicity figure, which expression is the same as the American method. The estimation of the amount of alkali salts can be carried out by the method of Woodroffe and Green (*J. S. L. T. C.*, 1922, **6**, 222).

The small amount of sulphur found in some chrome leathers has been shown by Innes (*J. S. L. T. C.*, 1919, **3**, 126) to be extracted with the fat by the petroleum spirit, and it can be estimated in the usual way after oxidation to sulphuric acid (*Leather Chemists' Pocket Book* (Procter), 1919).

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COLOURING MATTERS OF NATURAL ORIGIN

BY PROF. W. M. GARDNER, M. Sc., F. I. C.

The colouring principles from which natural colouring matters may be derived are very widely distributed in the vegetable kingdom. Natural colouring matters have been used by the natives of countries to which they are indigenous from very early times, and a large but diminishing number are still so used. Few important natural dyes are native to Europe, but a considerable number were imported in large quantities from the tropics prior to the introduction of the coal-tar dyes. The natural dyes have been replaced to a great extent by coal-tar derivatives, those now chiefly employed being indigo, logwood, fustic and Persian berries; but others still find considerable application for special purposes, and among these may be mentioned cochineal, orchil, madder, catechu, and turmeric.

Some natural dyestuffs, for example cochineal, are used without any previous preparation other than grinding. In other cases the natural product undergoes a simple preliminary treatment, as in the "ageing" of logwood; whilst the preparation of commercial indigo and of orchil involve a more complex treatment of the product formed by natural processes.

Few natural products are substantive dyes, and thus their application usually involves the use of mordants such as sodium or potassium dichromate, alum, sulphate of copper, etc. Certain commercial preparations, *e. g.* "single-bath logwood blacks" contain an admixture of such metallic salts.

The colouring matter present in a natural dye is usually associated in the commercial product with a considerable amount of tannin or other organic extractive matter, inert vegetable matter, mineral matter, etc. An exact estimation of the colouring matter is thus frequently a matter of great difficulty, and since the secondary

substances usually have an influence, adverse or otherwise, on the use of the dyestuff, a small scale experiment in dyeing or printing, carried out as far as practicable under the conditions in which the material will be used in practice, is often the most satisfactory means of estimating the commercial value of a natural dyestuff. Special methods are, however, available in certain cases, and such will be described under their appropriate headings.

Estimation of Colouring Matters by Means of Small-scale Dyeings

An exact estimation of the amount of pure colouring matter in a dyeing material of natural origin is often a very laborious and unnecessary process. What is usually required is the comparison of a sample with a standard, or of various samples amongst themselves, as regards strength, purity or money value. In such comparative dyeing experiments it is essential that the conditions of temperature, concentration and time of immersion, should be identical. Uniform temperature can best be assured by immersing the individual glass or porcelain dye-vessels in a common heating medium which may take the form of a shallow tank of copper or enamelled iron, in the top of which are four to six circular holes of suitable size to admit the dye-vessels. These rest on a perforated false bottom so placed that the dye-vessels can readily be lifted out. The tank may be heated by gas or by a closed steam coil and filled to the necessary depth with water for dyeing temperatures up to 80° or with concentrated solution of calcium chloride, glycerol or heavy mineral oil for higher temperatures. The volume of the liquors in the small dye-vessels must be kept uniform by adding distilled water to balance evaporation.

Wool, silk or cotton should be dyed in the experiments according to the use to which the dyestuff under test is to be put. Wool and cotton may be used either in skeins or as woven material but silk is most conveniently used in skeins.

In cases in which a large volume of boiling liquid relative to fibre, may be used, very accurately comparable results may be obtained by dyeing in ordinary glass flasks fitted with condensers; cutting the threads or woven material into small pieces which are kept in constant movement by the ebullition.

In practical dyeing operations the weight of liquid used rarely exceeds thirty times the weight of the material which is being dyed, but in small scale experiments this proportion is often inconveniently

small and this difference in concentration becomes important in the case of dye liquors which do not exhaust well.

Testing the Fastness of Dyeings

The term "fast dye" lacks precision, as there is no dyestuff which produces shades which are entirely unchanged by the various influences to which coloured materials are subjected. Used in this sense, therefore, the term fast is purely relative, but by convention it is applied to such dyestuffs as are reasonably resistant and thus fulfil practical requirements; the latter varying according to the nature and use of the material. For example, in the case of dyestuff used for colouring curtain material or carpets, fastness to light is of prime importance, fastness to washing being of quite secondary value. In other cases the conditions are reversed. The fastness of a given dyestuff usually varies on the different fibres, wool, silk and cotton.

The fastness properties of dyes have been the subject of much investigation. Owing to the fact that the first synthetic dyes produced were more fugitive to light than some of the natural dyes then used the idea was prevalent for a long period that the synthetic dyes were not "fast colours." But at the present time there is a far wider range of really fast dyes available than ever before. Hence the commercial possibility of a guarantee of "fadeless" colours. In the "Colour Index" issued in 1924 by the *Society of Dyers and Colourists* full details are given for the determination of the fastness of dyes to light, washing, milling, stoving, carbonising, rubbing and other influences. The fastness to each influence being recorded by the numerals 1 to 5; No. 1 representing "very fast."

The causes of the fading of colours under the influence of light are intimately connected with the constitution of the colour molecule and have been investigated without definite result. For the detection of dyes on the fibre see Analysis of Colouring Matters, Vol. 6.

A summary of the literature dealing with the action of light on dyes on cotton by P. W. Cunliffie will be found in *J. Soc, Dyers & Col.*, 1924, 40, 268.

INDIGO

Indigo has long been regarded as the most valuable and important of all dyestuffs. Certain species of indigo plant are found in most

tropical countries, and have been used by the natives of these countries as dyes or stains from prehistoric times. The largest amount of indigo is produced in the Indian provinces of Bengal, Oudh, and Madras, but it is also cultivated in China, Japan, Java, Manilla, Central America, Brazil, and certain parts of Africa. Before the introduction of synthetic indigo each of these countries exported its own special commercial brands, which were distinguishable by experienced buyers by their physical properties.

The indigo-yielding plants are not all of the same botanical family, but the most important commercial varieties are all species of the genus *Indigofera*. *I. Sumatrana* was the species chiefly cultivated in India, but some years ago it was extensively replaced by the Javanese plant *I. Arrecta*, from which a better yield was obtained for a period but the conditions for the successful continuous cultivation of the Java plant in India have never been worked out.

Isatis Tinctoria or woad plant is the European indigo plant, but is not now cultivated as a source of the dye, although it is still grown to some extent in England and Belgium, and, after preparation, used in the woad indigo vat to assist fermentation.

Natural indigo has been almost entirely replaced by the synthetic product. The decline dates from 1897, and in 1914 the amount used in all markets of the world was only about 5 per cent. of the quantity used in 1896. During the same period the area under indigo cultivation in India had decreased by seven-eighths and the number of persons employed in the industry by about nine-tenths. During the war period the cultivation temporarily increased four fold but has again steadily diminished.

It has recently been recognised that the historic Tyrian Purple dye produced from certain shell-fish is chemically closely related to Indigo. Both these products and many other derivatives of indigotin are now made synthetically and constitute the indigoid group of vat dyes.

Preparation of Indigo

The indigo plant is grown from seed each year, being cut down when the flowers begin to open, fresh shoots springing from the roots. The indigo-producing substance resides principally in the leaves.

Indigotin, the real colouring matter, does not exist in the plant, but is produced by the decomposition of a glucoside, *indican*, $C_{12}H_{12}O_6N$.

This was first isolated by Schunck and Roemer (*Ber.*, 1879, **4**, 2311) and was later obtained in a crystalline condition by Hoogewerff and ter Meulem (*Proc. K. Akad. Wetensch.*, 1900, **2**, 520). A laboratory method of preparing this substance from the plant in quantity has been described. (Perkin and Bloxam, *J. C. S. Trans.*, 1907, **91**, 1715).

To obtain indigo from the plant (see Rawson, *J. Soc. Dyers & Col.*, 1899, **15**, 168) the freshly cut plants are extracted with water in "steeping vats." Fermentation ensues, and the extracted indican is decomposed by a specific enzyme present in the plant. The liquid is then run into "beating vats" where it is agitated, and atmospheric oxidation changes the yellow colour of the liquid to green, and finally the indigo separates in flakes. The indigo pulp is collected, boiled with water to prevent secondary fermentations, and finally pressed into cubes or cakes and dried at a low temperature. Natural indigo is now placed on the market in the form of a 20 per cent. paste, to bring it into line as regard convenience with its synthetic substitute.

The quality and yield of indigo obtained depend greatly not only on the quality of the plant, selection of seed, manuring of crops, etc, but also on the skill brought to bear in the manufacturing process.

Associated with the blue colouring matter there is usually from 1 to 5% of a red colouring matter, *Indirubin*, which also is produced by the decomposition of indican. The amount of this red dye is increased by the addition of alkali to the steeping vats. Some brown amorphous product is also invariably present.

The amount of indigo produced from 100 pounds of fresh plant varies from 4 to 12 ounces.

Indican has been shown by Hazenwinkel (*Proc. K. Akad. Wetensch. Amsterdam*, 1900, **2**, 215) to be a glucoside of indoxyl, the sugar obtained from it being dextrose. The decomposition of indican in the steeping and beating vats is brought about by a hydrolytic enzyme first discovered by Schunck and later investigated by Beijerinck (*ibid.*, 1899, 120) and Hazenwinkel (*ibid.*, 1900, 513), the latter giving it the name *indimulsin*. The normal decomposition of indican results in the formation of indigotin by oxidation of indoxyl, but by combination of the latter with isatin, indirubin is formed.

Another secondary change results in formation of brown amorphous products by the condensation of indoxyl. The main brown product of this condensation has been named by A. G. Perkin (*J. C. S. Trans.*,

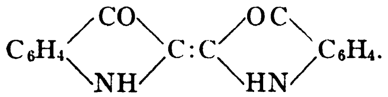
1907, **91**, 1728) *indoxyl brown* and has a percentage composition almost identical with that of the main constituent of indigo brown, which it closely resembles.

A substance allied to indican occurs under certain abnormal conditions in urine and gives rise to a blue coloration owing to the formation of indigotin.

The amount of indican in the leaves of the indigo plant may be estimated by decomposing the glucoside extracted from the leaves, by sulphuric acid and combining the indoxyl thus formed with isatin to form indirubin (Perkin and Bloxam, *J. C .S. Trans.*, 1907, **91**, 4), by oxidising the indoxyl to indigotin by means of ammonium persulphate (*ibid.*, 1907, **91**, 1728), or by condensing the indoxyl with *p*-nitrobenzaldehyde (Perkin and Thomas, *ibid.*, 1909, **95**, 795).

Constituents of Indigo

Indigotin, $C_{16}H_{10}O_2N_2$, has the constitution



and is the true colouring matter of indigo, from which it may be prepared in a variety of ways, but for analytical purposes it is best prepared from dry synthetic indigo. This is boiled with a 10% solution of pure sulphuric acid several times, then well washed with water. It is now reduced with sodium hydrosulphite in the presence of sodium hydroxide, the solution filtered, and the indigotin reoxidised by a current of air. The precipitate is collected, washed with dilute hydrochloric acid, then with alcohol, and dried. It is finally recrystallised from boiling glacial acetic acid, the crystals being washed successively with alcohol, ether, dilute hydrochloric acid, and lastly with water, and then dried at 105°.

An equally pure product may be obtained, according to Gaunt, Thomas, and Bloxam (*J. Soc. Chem. Ind.*, 1907, **26**, 1174), by sublimation. A flask containing the indigo is heated to a temperature of 420° in a bath of fusible metal, a high vacuum being maintained in the flask. The crystals of sublimed indigotin, which forms in the upper portion of the flask, are collected and finally purified by recrystallising from glacial acetic acid as above described.

Pure indigotin forms dark blue or purple needles which exhibit a coppery lustre. When powdered, it possesses a deep blue colour and assumes a bronzy lustre when rubbed. Its sp. gr. is 1.35. When heated, it sublimes at about 290°, the vapour possessing a beautiful red-violet colour, but in the presence of air considerable decomposition occurs. In a vacuum or an inert gas it volatilises unchanged. When submitted to dry distillation it decomposes; yielding aniline as chief product.

Indigotin is a neutral substance and is quite stable at ordinary temperatures; it has neither taste nor smell, and is insoluble in water, cold alcohol, ether, dilute acids, or alkalies, or fatty oils. It is slightly soluble in boiling alcohol with a blue colour, but is deposited again on cooling. It is also slightly soluble in phenol, carbon disulphide, or chloroform, but the best solvents are pyridine, glacial acetic acid, nitrobenzene, quinoline, naphthalene and aniline. The addition of 1 or 2 drops of sulphuric acid greatly increases the solvent action of glacial acetic acid, and from this solution the indigotin may be reprecipitated on dilution with water. Boiling paraffin dissolves indigotin with a magenta colour.

Concentrated sulphuric acid completely dissolves indigotin, sulphonation occurring. The product formed is either the mono-, di-, tri-, or tetra-sulphonic acid, according to the conditions.

When subjected to the action of acid oxidising agents such as dilute nitric acid, chromic acid, etc., in the presence of water, indigotin is converted into *isatin* ($C_8H_5NO_2$). With hot nitric acid it forms nitrosalicylic acid, $C_6H_3(OH)(NO_2)COOH$, or picric acid, $C_6H_2(OH)(NO_2)_3$, according to the conditions. Further oxidation products, such as oxalic acid and carbon dioxide, may be formed also.

If indigotin is heated with reducing agents in the presence of alkali hydroxide, it is reduced to *indigo white* ($C_{16}H_{12}N_2O_2$), but if the heating be long continued secondary changes take place, with the production of a dark red substance, $C_{32}H_{22}N_4O_4$, allied to *flavindine* ($C_{32}H_{24}N_4O_5$) (Giraud, *Bull. Soc. Chim.*, 1880, **34**, 530).

Indigo White, or Reduced Indigo ($C_{16}H_{12}N_2O_2$).—This substance, produced by the action of reducing agents on indigotin, is a greyish-white, amorphous, tasteless, and odourless substance. It is insoluble in water or dilute acids, but is soluble in alcohol, ether, and alkaline solutions, with a yellow colour. On exposure to air its solutions absorb oxygen, becoming at first green, and finally

blue, with reproduction and separation of indigotin. Alkaline solutions of indigo white give white precipitates with salts of aluminium, zinc, magnesium, or lead, and with stannous, ferrous, and manganous salts. It forms two compounds with lime, one soluble, the other insoluble in water.

The use of indigo in dyeing by the vat method is based upon the production of an alkaline solution of indigo white, with which the material is saturated, the indigo blue being then reproduced on the fibre by air oxidation.

Indigotin Sulphonic Acids.—The monosulphonic acid, $C_{16}H_9N_2O_2(SO_3H)$, also known as *sulphopurpuric acid*, is obtained by mixing 1 part of indigotin with 4 parts of concentrated sulphuric acid, and allowing the mixture to stand for half an hour. On diluting with water a fine purplish-blue precipitate is formed, which is slightly soluble in water. The sodium salt, which also is slightly soluble in water, has been used in dyeing under the name of "indigo purple," or "red indigo carmine."

Indigotin Disulphonic acid, $C_{16}H_8N_2O_2(SO_3H)_2$, also known as *sulphindigotic acid*, is produced by the further action of sulphuric acid, 1 part of indigotin being heated to 90° for half an hour with 10 to 12 parts of concentrated sulphuric acid. On diluting with water, any monosulphonic acid present is precipitated. The disulphonic acid may be purified by adding a saturated solution of common salt, which causes a precipitate of sodium disulphindigotate. It forms an insoluble lead compound, from which the free acid may be separated with hydrogen sulphide. It is easily soluble in water, and (like the monosulphonic acid) is rapidly destroyed by oxidising agents or converted into a leuco-compound by reducing agents. When treated with strong sodium hydroxide or ammonia, it produces first green, and then yellow substances, of which little is known.

Sulphindigotic acid is largely used in dyeing in the form of its sodium salt, which is known as "indigo carmine," or "indigo extract." It is sold as a bronze-blue powder or as a paste. Indigo extracts are estimated by dyeing trials or by titration with permanganate (see page 362).

Indigotin Tri- and Tetrasulphonic acids are produced by the action of fuming sulphuric acid on indigotin, the latter being formed by heating indigotin with fuming sulphuric acid (25% SO_3) for 20 minutes at 96–98° (Bloxam, *J. Soc. Chem. Ind.*, 1906, **25**, 736).

Both the di- and tetra-sulphonic acids are made use of in purifying commercial indigos for analysis.

Indirubin or Indigo Red, $C_{16}H_{10}N_2O_2$.—This substance, which is isomeric with indigotin, is produced in small amount (1 to 5%) in the ordinary process of manufacturing indigo. It is present in larger amount (up to 10%) in Java indigo and in Indian indigo made by the Coventry process. Like indigotin, it is formed from indican, a portion of the indoxyl produced by the hydrolysis being oxidised to isatin which then combines with indoxyl to produce indirubin, this change being facilitated by the presence of alkali. When pure, indirubin forms brownish-red needles, which sublime at 140°. Like indigotin it is insoluble in water, alkali, or dilute acids, but is much more soluble in alcohol than indigotin, and is also somewhat readily soluble in (commercial) acetone or in pyridine. Concentrated sulphuric acid converts it into a disulphonic acid. Toward reducing agents it behaves similarly to indigo blue, being converted into a compound analogous to indigo white, which is re-oxidised when exposed to the air. It is much less susceptible to the action of oxidising agents than indigotin, and when a mixture of the sulphonic acids is titrated with potassium permanganate, the whole of the indigotin disulphonic acid is destroyed before the indirubin disulphonic acid is attacked. For the same reason, commercial indigo containing indigo red is unsuitable for producing white discharge patterns in calico printing.

Indirubin produces crimson shades in dyeing by the vat method, but if the vat contains an excess of alkali, the indirubin white compound undergoes an intramolecular change and is slowly converted into indigotin white, which on oxidation produces indigo blue.

Indirubin sulphonic acid dyes wool a crimson shade, which is much faster to light than the ordinary indigo-extract dyes.

Indigo Brown.—Brown substances are always present in commercial indigo. They were named by Schunck, *indiretin* and *indihumin*, and are formed by secondary decompositions of indican. The brown matter is soluble in sodium hydroxide solution and in concentrated sulphuric acid.

Rawson states that the longer the liquid is allowed to stand after extraction from the plants before oxidising, the greater the amount of brown produced at the expense of indigo blue (*J. Soc. Chem. Ind.*, 1907, **26**, 279). Perkin and Bloxam (*J, C. S. Trans.* 1907, **91**, 279)

have isolated three substances from indigo brown and have obtained figures for the main constituent corresponding with the formula $C_{16}H_{12}O_3N_2$. They found pyridine to be the only practical solvent, and considered that indigo brown is derived by the condensation of indoxyl produced from indican in the steeping vats.

Indigo Yellow.—This substance is frequently present in commercial indigo, particularly Java indigo (Rawson, *J. Soc. Chem. Ind.*, 1899, **18**, 251). It is almost insoluble in water or in dilute acids, but is soluble in concentrated sulphuric acid or in glacial acetic acid. It is easily soluble in alkalies, from which solutions dilute acids throw down a pale yellow precipitate. It is soluble in alcohol and ether. It sublimes at a low temperature with much decomposition; it dyes wool mordanted with potassium dichromate a yellow shade resembling that given by weld. It does not dye in the vat.

A. G. Perkin (*J. Soc. Chem. Ind.*, 1907, **26**, 435) has shown that this substance is identical with *kæmpferol*, $C_{15}H_{10}O_6$. Indigo yellow is liable to cause errors in indigo estimations unless previously eliminated.

Indigo Gluten.—This substance is present in varying amounts (up to 20 %) in indigo, from which it was first isolated by Berzelius. Richardson, Wood and Bloxam (*J. Soc. Chem. Ind.*, 1907, **25**, 4) prepared it as a horny mass which evolved ammonia on heating. It is soluble in water but exists in indigo as an insoluble metallic (calcium?) compound.

Synthetic Indigo.—The synthesis of indigo was first completed by Baeyer in 1878, *o*-aminophenylacetic acid being converted into indoxyl, from which isatin was formed. The conversion of this substance into indigotin had been previously achieved by Baeyer and Emmerling in 1870.

Somewhat later Baeyer prepared indigotin from cinnamic acid, and in 1882, in conjunction with Drewsen, he published a new synthesis, in which indigotin was obtained from *o*-nitrobenzaldehyde by treatment with acetaldehyde in the presence of alkali hydroxide.

In 1893 Kalle & Co. put on the market a product named "indigo salt," which is the sodium bisulphite compound of *o*-nitrophenyllactone. This was used to a limited extent in calico-printing.

In 1890 Heumann's synthesis from phenylglycocol was published, and this was gradually developed into a commercially successful process by the Badische Anilin und Soda Fabrik, who placed their product, "Indigo Pure" on the market in 1897.

Synthetic indigo is now manufactured on an enormous scale in this country as well as in Germany, France, Switzerland and the United States.

Indirubin has also been produced synthetically, and has been put on the market both as an independent product and admixed with indigotin as in natural indigo. It is not much employed.

An interesting account of the history of the development of the manufacture of synthetic indigo was published in the *Berichte* by H. Brunck in October, 1900 (*J. Soc. Dyers and Col.*, 1901, **17**, 157).

Synthetic indigo is placed on the market as a blue powder of about 98% purity and as a paste containing 20% of indigotin. The chemical and dyeing properties of the product are identical with those of indigotin from natural indigo.

Commercial Varieties of Indigo

Commercial varieties of natural indigo differed widely in appearance, character, amount of impurities, percentage of colouring matter, proportion of indigo red, etc. and were distinguished by names indicating their origin, *e. g.*: Bengal, Java, Guatemala, Caracas, Madras, Manilla, Oudh, Egyptian, etc.; and by long experience buyers became very expert in distinguishing samples by consideration of their physical characteristics, such as colour, weight, porosity, friability, appearance when rubbed, etc.

Analysis of Indigo.—The useful constituent in commercial indigo, indigotin, varies in amount in different samples from 30 to 80%. The only other useful constituent is indirubin, which is present in amount varying from less than 1% to 10%. There is also present indigo-brown, indigo yellow, indigluten, with other organic impurities, moisture, and mineral matter.

Moisture.—0.5 grm. of the finely powdered sample is dried to constant weight at a temperature of 105°.

Mineral Matter.—1 grm. of the finely ground sample is carefully ignited. In the case of pure qualities the ash is frequently as low as 3%, but in inferior kinds the amount may reach as much as 25 to 30%.

Indigotin.—Many different methods have been proposed for estimating the percentage of indigotin in commercial indigo. They may be conveniently classified as follows:

(*a*) Colorimetric tests.
(*b*) Comparative dyeing trials.
(*c*) Extraction by solvents.
(*d*) Sublimation.
(*e*) Oxidation tests.
(*f*) Reduction tests.
(*g*) Nitrogen estimation.

Of these only (*e*) and (*f*) need be considered in any detail, but short reference will be made to the other methods.

(*a*) Colorimetric Tests

Equal weights of pure indigotin (prepared as on p. 354) and of the sample under examination are dissolved in sulphuric acid. The sulphindigotic acid is purified (see oxidation methods, p. 363) and the relative intensity of colour of the solutions estimiated in a Dubosc colorimeter or Lovibond tintometer. This method of estimation is liable to inaccuracies due to coloured impurities in the natural indigo, which are difficult to remove. It is also affected by any difference in amount of indigo red in the standard and in the sample. C. H. Wolff (*J. Soc. Chem. Ind.*, 1884, **3**, 156) states that this difficulty can be avoided by observing the absorption-spectrum (1 in 800,000).

(*b*) Comparative Dyeing Trials

The samples of indigo are dissolved in sulphuric acid and made into standard solutions (see oxidation methods, page 363) containing 1 grm. of the sample per litre. 10 grm. of wool are then dyed with 500 c.c. water containing 50 c.c. of the solution with the addition of 2 c.c. of 10% sulphuric acid. The dyed patterns are compared with standard patterns dyed in a similar manner with similar solutions of pure indigotin, (see p. 354).

Grossmann (*J. Soc. Dyers and Col.*, 1897, **13**, 124) proposes a special form of apparatus for carrying out these tests.

Dyeing trials are subject to the same inaccuracies as colorimetric tests.

(*c*) Extraction by Solvents

A large number of processes for estimating indigotin have been based on the extraction of the indigotin by volatile solvents.

Extraction by Aniline.—Hoenig's process (*Zeitsch. angw. Chem.*, 1889, **2**, 10) is as follows: 0.8 gm. of indigo is mixed with 2.5 grm. finely powdered dry pumice stone. The mixture is extracted with 50 c.c. of aniline oil in a Zulkovsky-Wolfbauer apparatus for 1 hour. The mass is then removed, washed with alcohol, dried, powdered, returned to the apparatus, and extracted a second time. The mixed extracts are evaporated on an oil bath to about 10 c.c. then mixed with 50 c.c. of absolute alcohol. The precipitated indigotin is collected on a weighed filter, washed with alcohol, dried at 110°, and weighed. The method is not very accurate.

Brandt (*Rev. Gén. Mat. Col.*, 1897, **1**, 43) similarly extracts with aniline oil in a Soxhlet apparatus. After extraction the aniline is removed by treatment with dilute hydrochloric acid and the indigo collected on a tared filter, washed with boiling water, then with cold alcohol, dried, and weighed.

Extraction by Phenol.—Brandt (*Rev. Gén. Mat. Col.*, 1898, **2**, 26) later states that extraction with aniline destroys a portion of the indigotin, and proposes to use 30 grm. of phenol for 0.2 grm. indigo. The extraction is complete in half an hour. After cooling, a solution of 20 grm. of sodium hydroxide in 250 c.c. water is added, the indigo is collected on a tared filter, washed with boiling water until neutral, then with alcohol, and dried.

Extraction by Naphthalene.—Schneider (*Zeitsch. anal. Chem.*, 1895, **34**, 347) extracts 1 grm. indigo with 50 grm. boiling naphthalene, until the drops which fall from the extractor are colourless. After cooling, the indigotin is precipitated by adding ether, then collected, washed with ether, dried, and weighed. The extraction occupies 5–6 hours, and a certain amount of destruction of indigotin occurs, for which a correction amounting to 0.1–0.4% is made. The whole of the materials and apparatus must be carefully dried to avoid the danger of explosion.

Extraction by Nitrobenzene.—Gerland (*J. Soc. Chem. Ind.*, 1896, **15**, 17; 1897, **16**, 108) has devised a simple apparatus for extracting indigo with nitrobenzene. 0.5 grm. indigo is placed in a filtering tube and extracted with 25 c.c. nitrobenzene for 1 hour. The indigotin separates in beautiful crystals, only a small proportion remaining in solution; to save the trouble of recovering this, nitrobenzene saturated with indigotin in the cold is used for the extraction. The indigotin is collected on a tared filter, washed with benzene, and

dried. Although apparently pure, the crystals contain 3–6% impurity, and a prolonged treatment with hydrochloric acid containing a little hydrogen peroxide is necessary before weighing.

Extraction by Acetic Acid.—Brylinski (*Rev. Gén. Mat. Col.*, 1898, 2, 52) extracts 0.2 grm. of indigo with 50 c.c. glacial acetic acid in a Soxhlet flask, using a thimble filter. The acetic acid is boiled over a free flame, and the extraction continued until the solvent passes through colourless. After cooling, the acetic acid is diluted with four times its volume of water, which precipitates the indigotin in flakes. The liquid is passed through a tared filter and the indigotin washed first with boiling water, then with alcohol, finally with ether, dried and weighed.

Extraction of the Impurities by Solvents.—Methods have been suggested based on the extraction of the organic and mineral impurities by a succession of solvents, but no useful results have been published.

(*d*) Sublimation of the Indigotin

Satisfactory quantitative results cannot be obtained by this process.

(*e*) Oxidation Process

In these processes the indigotin is first converted into a sulphonic acid, which is purified, made into a standard solution, and a portion then titrated with a standard solution of some suitable oxidising agent such as potassium permanganate; the oxidising agent being itself standardised by means of a solution of pure indigotin.

The process involves four stages; 1. preparation of the sample; 2. dissolving the indigotin in sulphuric acid; 3. purification of the sulphonic acid; 4. titration with the standard oxidising agent. This method of estimating indigotin was originally developed by Rawson, who devised the permanganate method (*J. Soc. Dyers and Col.*, 1885, 1, 74).

1. Preparation of the Sample.—Since various chests of the same manufacture or even various lumps in the same chest of indigo may differ in percentage of colouring matter, as great a variety of samples as possible should be obtained from the bulk. These should be coarsely ground, well mixed, and a small portion finely ground to an inpalpable powder. Moisture should then be estimated by drying at 105°, and the dried sample used for Process 2.

2. Dissolving the Indigo in Sulphuric Acid.—Rawson mixes 0.5 grm. of the finely powdered indigo with its own weight of ground glass in a small mortar. The mixture is gradually added to 20 c.c. of concentrated sulphuric acid contained in a cylindrical porcelain crucible (capacity 30 c.c.) constantly stirring with a glass rod. The mortar is rinsed with a little powdered glass which is added to the mixture and the crucible is heated for 1 hour in a water oven at 70°. Under these conditions indigotindisulphonic acid is exclusively formed. The mass is diluted with water, made up to 500 c.c. and filtered to remove the glass and certain insoluble impurities.

In place of the crucible, Schulten prefers the use of small glass-stoppered bottles which can be readily shaken, and recommends that the sulphonation be carried out for 15 minutes at the temperature of the boiling water bath.

Bloxam (*J. Soc. Chem. Ind.*, 1906, **25**, 735) recommends that the indigotin be converted into the tetrasulphonic acid, which eliminates the necessity for subsequently purifying the sulphonic acid by salting out as described in Process 3. 1 grm. of indigo is mixed with 2–3 grm. of purified sand, and placed in a 1-oz. spouted beaker. 5 c.c. of fuming sulphuric acid (25% SO_3) are added and the mixture stirred with a glass rod. The beaker is heated in the water oven for half an hour, then cooled, and the contents diluted to 500 c.c. 100 c.c. of this solution are mixed with 80 c.c. of a solution containing 450 grm. potassium acetate per litre. The mixture is warmed until the precipitate which is first formed redissolves. The solution is then cooled and left for 1 hour in a vessel containing ice water. The potassium tetrasulphonate which crystallises out is collected in a Gooch crucible and washed with an ice cold solution containing 225 grm. potassium acetate and 12 c.c. glacial acetic acid per litre. The precipitate is finally dissolved in hot water, prolonged heating being avoided, and the solution made up to 500 c.c. Wanjerin and Vorlander (*Zeit. Farb. Tex. und Chemie* 1902, 281) state that using 8% fuming sulphuric acid at 95°–100° for half an hour a loss of 2% indigotin occurs through oxidation but Bloxam denies this.

3. Purification of the Disulphonic Acid.—Several of the impurities contained in natural indigo are rendered soluble by the sulphuric acid treatment, thus appearing in the final solution, and reacting with potassium permanganate or other oxidising agent,

introduce errórs into the estimation. Various methods of purifying the indigotin disulphonic acid have, therefore, been suggested.

Salting-out Method.—50 c.c. of the filtered solution of indigo are mixed with 50 c.c. of water and 32 grm. of salt. The liquor which is thus almost saturated with salt is allowed to stand for 1 hour. The precipitated indigotin-sodium-disulphonate is collected and washed with about 50 c.c. of saturated salt solution. It is then dissolved in water, cooled, mixed with 1 c.c. sulphuric acid, diluted to 300 c.c. and titrated as described under 4. A correction, amounting to 0.001 grm., is required in order to allow for the small quantity of sodium indigotindisulphonate which dissolves in a saturated solution of common salt.

Barium Chloride Process (Rawson, *J. Soc. Chem. Ind.*, 1899, **18**, 251).—After dissolving the indigo in sulphuric acid and diluting with water, but before making up to 500 c.c., 10 c.c. of a 20% solution of barium chloride are added. The solution is then diluted to 500 c.c. and well mixed. On standing, the barium sulphate formed subsides and carries down with it the suspended impurities. In 15 to 20 minutes the clear solution may be withdrawn from the top of the flask for titration.

Calcium Carbonate Process (Grossmann, *J. Soc. Chem. Ind.*, 1905, **24**, 308).—About 6 grm. of pure calcium carbonate is employed instead of the barium chloride in the last described process.

Bergtheil and Briggs (*J. Soc. Chem. Ind.*, 1906, **25**, 729) state that both barium chloride and calcium carbonate cause a precipitation of colouring matter, and suggest the use of freshly precipitated barium sulphate. According to these authors precipitation either with salt or barium chloride gives satisfactory results.

Donath and Strasser (*J. Soc. Chem. Ind.*, 1894, **13**, 426) propose to remove the impurities by extracting the ground indigo in a Soxhlet apparatus with dilute hydrochloric acid, hot water, and finally with alcohol and ether, before solution in sulphuric acid.

4. **Titration with Standard Oxidising Solution.**—Many oxidising agents have been suggested for titrating indigo, but potassium permanganate solution is now universally preferred, as with other reagents, *e. g.* potassium dichromate. The end-point is less sharply defined.

Rawson recommends the following procedure: 50 c.c. of the sulphindigotic acid solution, after purification by one or other of the processes

described under (3), are diluted to 300 c.c. with distilled water and placed in a white porcelain dish. A solution of N/50 potassium permanganate (0.632 grm. per litre) is gradually run in from a burette until the liquor which at first becomes green, changes to a light yellow colour. With pure indigotin the end-point is quite sharp, but with low qualities of indigo some practice is necessary in order to obtain concordant results. The end-point is also more difficult to determine in the case of indigos containing notable quantities (more than 1%) of indigo red. In such cases, the indirubin must be separately estimated by the process described below.

The indigotin factor of the permanganate solution is obtained by dissolving 0.5 grm. of pure indigotin prepared as described on page 354 in sulphuric acid, diluting and titrating as above described.

It is essential that the prescribed conditions should be closely adhered to throughout.

The oxidation of the indigotin (as sulphonic acid) by the permanganate is represented by the equation $5C_{16}H_{10}N_2O_2 + 4KMnO_4 + 6H_2SO_4 = 10C_8H_5O_2N + 2K_2SO_4 + 4MnSO_4 + 6H_2O$.

According to this equation 4 molecules (316 parts) of potassium permanganate are equivalent to 5 molecules (565 parts) of indigotin, but experiment shows that at the dilution necessary to obtain a satisfactory end-point the permanganate factor is somewhat lower than theory requires, and each 1 c.c. of N/50 permanganate = 0.00147 grm. of pure indigotin.

Analysis of Indigo Rich in Indirubin.—The analysis of samples of indigo containing more than about 1 per cent. of indirubin by the ordinary permanganate process is not easy.

As already stated, indirubin is more resistant to the action of oxidising agents than indigotin. Both substances are sulphonated and appear together in the solution to be titrated. As the permanganate is added, the blue colour due to the indigotin is first destroyed and the liquid which first assumes a dirty green or brownish colour changes to crimson when the whole of the blue is oxidised. Further additions of permanganate eventually destroy the indirubin, but there is an obvious difficulty in distinguishing between the crimson colour due to indirubin and the very similar colour due to excess of permanganate. The end-point is thus frequently a matter of much uncertainty.

In the case of fairly pure indigo, practice will, however, enable an approximate estimation of indigotin and indirubin by direct titration with permanganate. Other oxidising agents, such as potassium dichromate, are less suitable for the titration of indigo as the end-point is less sharply defined.

Usually, however, it is more satisfactory to extract the indirubin from the sample before sulphonating.

Koppeschaar (*Zeitsch. anal. Chem.*, 1899, **38**, 1) extracts the indigo with glacial acetic acid, and estimates the indirubin colorimetrically against a standard solution of indirubin in the same solvent.

Rawson (*J. Soc. Chem. Ind.*, 1899, **18**, 252) uses ether and proceeds in a similar manner.

Gardner and Denton (*J. Soc. Dyers and Col.*, 1901, **17**, 170) made an investigation with various solvents and state that ether when pure has little solvent action on indirubin. They recommend commercial acetone as the most satisfactory solvent. 0.2 grm. of the finely powdered and dried sample is boiled for half an hour with 100 c.c. commercial acetone using a reflux condenser. After cooling, the solution is diluted to 200 c.c. with a 10% salt solution, which precipitates the small amount of indigotin dissolved and also some brown impurities. After shaking, the solution is allowed to stand for 5 minutes, filtered, and the indirubin estimated colorimetrically by comparison with a standard solution of pure indirubin prepared with acetone and salt solution in the same way.

Bloxam and Perkin (*Trans.*, 1910, **97**, 1460) recommend pyridine as the best extractive agent: 0.25 grm. of the finely sieved and dried indigo is mixed with 25–30 grm. of purified sand and placed in a thin-walled glass tube (the "container"). This is closed at one end by a piece of cotton cloth fastened round with silk cord (wire must not be employed). A layer of asbestos is first placed in the container and the sand and indigo mixture is poured in. The upper surface of the indigo mixture is covered with a layer of sand and loose asbestos. The container is placed in a Soxhlet tube so as to rest on 2 or 3 glass marbles to facilitate drainage. The tube is then extracted with pyridine for about half an hour or until the liquid possesses the blue colour of pure indigotin. The pyridine extract is distilled to a small bulk treated with boiling water and again distilled until the last traces of pyridine have disap-

peared. On cooling, the indirubin and indigotin, together with some indigo brown, are precipitated, collected in a Gooch crucible lined with asbestos, and treated with hot 15% hydrochloric acid to decompose any calcium salt of indigo brown, then washed with a hot 10% sodium hydroxide solution which completely dissolves the brown impurity. The product is finally treated with 1% acetic acid, the crucible placed in a small beaker, and after drying, 5 c.c. of pure sulphuric acid are added. On heating the mixture for half an hour, both indigotin and indirubin are sulphonated. The mixed sulphonates are dissolved in hot water, filtered, and made up to 250 c.c. The quantity of each colouring matter present is then estimated colorimetrically by comparison with standard mixtures of pure indigotin and indirubin sulphonic acids. The best dilution is usually about 5 c.c. of the above solution in 200 c.c. of water, but with small amounts of indirubin a greater concentration is necessary.

Since only a portion of the indigotin is extracted from the original sample, the remainder is sulphonated and estimated either colorimetrically or by means of potassium permanganate.

Analysis of Indigo Containing Yellow Colouring Matter.—It has already been stated (page 358) that many samples of indigo contain a yellow colouring matter, *kœmpferol*, and this interferes with the permanganate and with many other processes of estimation. Before testing, it is desirable therefore to determine whether this substance is present. A small quantity of the powdered indigo is sprinkled on the surface of a few c.c. of ammonia in a porcelain dish, when, if indigo yellow is present, a yellow solution is obtained.

When this occurs, the weighed amount of the sample is treated with a warm dilute solution of ammonia (or with alcohol or ether) to remove the yellow, then collected on an asbestos filter, washed, dried, and dissolved in sulphuric acid in the usual way.

Analysis of Indigo Containing Starch.—Thompson (*J. Soc. Dy. and Col.*, 1911, **27**, 49) has shown that the titration with permanganate by either Rawson's or Bloxam's method is quite inaccurate when indigo contains starch. Frank and Perkin (*J. Soc. Chem. Ind.*, 1912, **30**, 372) confirmed this but state that correct results are obtained if the starch is previously removed by boiling the indigo with a 4 per cent. solution of hydrochloric acid.

(*f*) Reduction Tests

There are two methods of estimating indigotin by the use of reducing agents:

1. The indigo is sulphonated as in the case of oxidation tests, and then titrated with a standard reducing solution.
2. The finely ground indigo is treated direct with a suitable reducing agent in the presence of alkali, under which conditions the blue colour of the indigotin disappears and indigo white is formed.

The amount of indigotin may be estimated either (*a*) by noting how much of a standard solution of the reducing agent is required to completely destroy the blue colour of the indigotin or (*b*) by completely reducing the indigotin to indigo white, then re-oxidising, collecting, and weighing the indigotin.

Reduction of Sulphonic Acids.—Müller (*Ber.*, 1888, **13**, 2283) reduces indigotindisulphonic acid by means of a standard solution of sodium hydrosulphite ($Na_2S_2O_4$). This process is suitable only when a long series of estimations have to be made, since the apparatus required is somewhat complicated. The process is, however, capable of great accuracy, and is carried out as follows:

Sodium Hydrosulphite Process.—The solution is prepared by dissolving about 3 grm. of solid hydrosulphite powder with the addition of 2 grm. sodium hydroxide, in a litre of water.

It is standardised by means of pure indigotin or ammoniacal copper sulphate, using indigotindisulphonic acid as indicator in the latter case. The standard solution is prepared to contain either, 1 grm. of pure indigotin (converted into disulphonic acid) per litre or 1.904 grm. of pure crystallised copper sulphate + 100 c.c. strong ammonia per litre. These solutions are equivalent.

50 c.c. of the standard solution of indigotin are placed in a wide-mouthed 200 c.c. flask fitted with an india-rubber stopper perforated with 3 holes. Into one hole is fitted the tip of a glass-stoppered burette containing the hydrosulphite solution. The other two holes serve for the entry and exit of a current of coal gas, since it is essential that the process should be conducted in the absence of air. Air must also be excluded from the hydrosulphite burette, the upper portion being supplied with a neutral atmosphere by suitable means. It is also connected with a stock bottle containing a further supply of hydrosulphite.

The flask is boiled to expel air, and then allowed to cool, coal gas being admitted. The solution of hydrosulphite is now gradually run in until the indigotin is just decolourised. Under the conditions named, each c.c. of hydrosulphite used corresponds to $\frac{0.05 \text{ grm.}}{n.}$ indigotin, n indicating the number of c.c. of hydrosulphite used.

If ammoniacal copper sulphate is used for standardising the hydrosulphite, the cork should be provided with a fourth hole, in which is placed a second burette containing indigo solution to use as indicator. A few drops of this are added when the copper solution is almost decolourised, in order that a sharp end-point may be obtained. In other respects the process is carried out exactly as above described.

The titration of the sample is carried out in the same way as used in standardising the hydrosulphite. 0.5 grm. of the sample of indigo is dissolved in sulphuric acid and made up to 500 c.c. as in the permanganate process, 50 c.c. being then titrated with hydrosulphite until decolourised.

In a sample of indigo containing much indigo red, the indigotin becomes first decolourised, and at this stage the liquid assumes a reddish-violet colour. By further titration, the indirubin is attacked and a yellow solution is obtained. In this way the amount of each of the two colouring matters can, with practice, be approximately ascertained.

In the case of pure qualities of indigo, the fully reduced liquid is of a pale yellow colour, but with low qualities it finally assumes a brownish-yellow appearance.

Binz and Kufferath (*Färb. Ztg.*, 1903, 225) recommend that the titration with hydrosulphite be carried out *in vacuo* instead of in the presence of an inert gas.

Gerland (*J. Soc. Chem. Ind.*, 1896, **15**, 15) prefers to convert the indigotin first into monosulphonic acid, from which impurities are separated by filtration through sand. The monosulphonic acid is then precipitated by addition of water, collected, dried, and converted into the disulphonic acid, which is then titrated with hydrosulphite solution.

Titanous Chloride Method.—Knecht (*J. Soc. Dyers and Col.*, 1904, **20**, 97; 1905, **21**, 292), who has worked out this process, states that it has the advantage that titanous chloride is much more stable than sodium hydrosulphite. 50 c.c. commercial titanous chloride (20%

solution) and 50 c.c. strong hydrochloric acid are boiled together, cooled, and made up to 2 litres. This solution is standardised by means of pure indigotin or ferric iron, 262 parts of indigotin corresponding to 112 of iron (Fe). Knecht found that the impurities present in natural indigos obscure the end-point, and recommends Grossmann's method of purification with calcium carbonate. The process is carried out as follows:

1 grm. of indigo is sulphonated with 5 c.c. of 100% sulphuric acid at 90° for 1 hour. The solution is diluted, poured into a 500 c.c. flask, 12 grms. of chalk are slowly added, and after the evolution of carbon dioxide has ceased the liquid is cooled and made up to 500 c.c. 50 c.c. of the clear solution are run into a flask, and 25 c.c. of a 20% solution of Rochelle salt added. The flask is provided with an india-rubber stopper containing 3 holes, two for entry and exit of carbon dioxide and the third for the burette containing the titanous chloride. After the air has been displaced the titration proceeds until the blue colour changes to yellow. This process gives very satisfactory results.

Gravimetric Reduction Process.—The earliest methods for the estimation of indigo were based on the formation of a small vat from which, after complete reduction, a measured quantity of the solution was withdrawn, the indigotin reprecipitated by oxidation, collected, washed, dried and weighed.

Many processes based on this general idea have been proposed. The following, due to Rawson, gives satisfactory results:

1 grm. of finely powdered indigo mixed to a paste with water is placed in a litre flask with 500 c.c. of lime water. The flask is furnished with an india-rubber stopper containing 4 holes, two for the entry and exit of a current of coal gas, one carrying a siphon, and the fourth a stoppered funnel. The liquid is heated to about 80° then coal gas is admitted and about 250 c.c. of a solution containing 3 grm. solid hydrosulphite is run in through the funnel. The liquid, which assumes a yellow colour, is kept hot for half an hour, and after allowing the insoluble matters to deposit, 500 c.c. of the clear liquid is run off into a flask, and air is drawn through for about 20 minutes to re-oxidise the indigo white to indigotin. About 10 c.c. of hydrochloric acid isthen added and the liquid heated nearly to boiling. The precipitate is collected on a tared filter, washed with hot dilute hydrochloric acid, then with water, dried, and weighed.

The remaining solution in the original flask is measured and the amount of indigotin in the total calculated. Greater accuracy is obtained by dissolving the recovered indigotin in sulphuric acid and titrating with permanganate.

H. M. Rau (*J. Amer. Chem. Soc.*, 1885, **7**, 16) uses grape-sugar and sodium hydroxide in forming his vat, and otherwise proceeds as above.

F. A. Owen (*J. Amer. Chem. Soc.*, 1888, **10**, 24) recommends a vat containing zinc dust and ammonia.

G. Engel proposes the use of vanadyl sulphate. 10 grm. of ammonium vanadate are dissolved in 100 grm. of concentrated sulphuric acid with the aid of heat. The red solution obtained is poured into 2 litres of water at 50°. To this are added 50 grm. zinc powder. The purplish-blue solution is filtered and cooled. The titration is carried out exactly as with hydrosulphite.

(*g*) Nitrogen Estimation

F. Voeller (*Zeits. Farb. Text. Chem.*, 1891, **1**, 110) proposed to estimate indigotin by estimating the nitrogen content by Kjeldahl's method, the nitrogen found when multiplied by the factor 9.36 giving the indigotin. It is obviously necessary to completely remove all nitrogenous impurities from natural indigo before carrying out this method, and this is found to be impracticable.

Other Methods of Analysis.—Möhlau and Zimmermann (*Zeit. Farb. Text. Chem.*, 1903, **3**, 189) convert indigotin into the monosulphonic acid by heating 0.1 grm. of the finely ground sample for 15 minutes with 50 c.c. of a mixture of 100 c.c. glacial acetic acid and 4 c.c sulphuric acid. The solution is filtered hot and the residue washed with the warm acid mixture, until the filtrate is colourless. The filtered solution is then heated to 70° and poured drop by drop into 100 c.c. of boiling water. This hydrolyses the monosulphonic acid, indigotin being reproduced. The process is not satisfactory, since the final product is not pure.

Estimation of Indigo on the Fibre

Vat-dyed indigo is found on all materials, cotton, wool, and silk.

An exhaustive paper dealing with the estimation of indigo on dyed wool materials has been published by Green, Gardner, Lloyd and Frank (*J. Soc. Dyers and Col.*, 1913, **29**, 227–241). It comprises:

I. a critical examination of all previously published methods; II. a description of new methods for the quantitative estimation of indigo by weight; and, III. methods for the estimation of the proportion of the total depth of shade which is due to indigo when other dyestuffs have been used in conjunction with it.

I. Examination of Known Methods of Analysis.—With the object of submitting these methods to critical investigation, instead of indigodyed materials, pieces of undyed woollen cloth were used, or woollen cloth dyed with colouring matters usually employed for topping or bottoming indigo, in which were wrapped weighed quantities of pure indigotin. Such cloth was then submitted to the extraction methods recommended by the various authors, and the recovered indigo either weighed as such or submitted to sulphonation with concentrated sulphuric acid at 70° and estimated by titration with N/50 potassium permanganate.

Rawson's Hydrosulphite Method.—The indigo is separated from the cloth by reduction with an alkaline solution of sodium hydrosulphite, precipitated from the solution by aeration, filtered off, and weighed or estimated volumetrically, after sulphonation, by titration with permanganate.

The indigo separated by this method is apparently very pure, but the process is tedious, a large volume of liquid having to be filtered. The method gives moderately good results with lightly dyed materials, but somewhat variable results with heavily dyed, thick, felted cloths.

The method has been compared by Binz and Rung (*Zeit. angew. Chem.*, 1898, 904) with the acetic acid extraction method (which follows), and they find that the latter is less troublesome and more rapid, whilst the results are somewhat higher.

Brylinski's Method.—Brylinski (*Rev. Gén. Mat. Col.*, 1898, **2**, 54; 1899, 5; *J. Soc. Dyers and Col.*, 1898, **14**, 75) extracts the material in a Soxhlet apparatus with glacial acetic acid, afterwards diluting the solution with water, filtering off the precipitated indigo on a weighed filter, washing with alcohol and ether, and finally drying and weighing. The method was improved by Binz and Rung (*loc. cit.*), who dilute the acetic acid extract with a smaller quantity of water and mix in a separating funnel with ether. The indigo becomes suspended in the ether, leaving the aqueous acetic acid layer almost clear. The indigo can then be readily separated from the

ether by filtration through a hardened filter paper, washed with alcohol and ether, dried, and weighed. Binz and Rung have found that a portion of the indigo is decomposed during the long boiling necessary for the extraction, and this they attribute to the reducing action of the wool. Green and his co-workers however have proved that the loss is due to the decomposition by heat of indigo which has crystallised out upon the sides of the boiling flask.

The use of paper, even parchmentised paper, for filtration was found objectionable, 1. on account of its liability to vary in weight, and 2. because the precipitated indigo cannot be subjected to sufficiently rigorous treatment with reagents to remove impurities.

Möhlau and Zimmerman's Method.—(*Zeit. Färb. Text. Chem.*, 1903, 189.) In this method, 10 grm. of the material, which is cut as fine as possible, are heated in a flask or beaker, on a rapidly boiling water-bath, with 100 c.c. of acetic-sulphuric acid (100 c.c. glacial acetic acid and 4 c.c. concentrated sulphuric acid) for about half an hour, shaking occasionally. The hot solution is filtered through a Gooch crucible, using hardened filter paper, the residue being repeatedly heated on the water-bath with acetic-sulphuric acid and filtered until the filtrate is no longer blue. The extract is warmed to 50° to redissolve the indigo, and is then diluted to twice its volume with boiling water. After cooling, the indigo is filtered off on a weighed, hardened filter paper, well washed with hot water until the filtrate is no longer acid, then with a little alcohol, and finally with 100 c.c. of ether, dried at 110°, and weighed. From the percentage of indigo obtained there is deducted for cotton materials 0.22%, this being the amount of modified cellulose supposed to be present with the indigo. With woollens no correction is considered necessary, since it is assumed that the dissolved wool remains in solution on dilution.

This method gives very variable results, and the difficulty of manipulation is greatly increased when certain topping or bottoming colours are present, *e. g.*, logwood, myrobalans, etc.

Other Extraction Methods.—The extraction of indigo from the fibres by solvents has generally been carried out in a Soxhlet apparatus, and the following substances, in addition to acetic acid, have been proposed for the purpose: phenol, aniline, (Koenig, *Zeit. f. angew. Chem.*, 1899, **2**, 10) naphthalene, (Schneider, *Zeitsch. anal. Chem.*, 1895; *J. Soc. Dyers and Col.*, 1895, **11**, 194) and nitrobenzene,

(Gerland, *J. Soc. Chem. Ind.*, 1896, **15**, 17; 1897, **15**, 100). In using these solvents for extracting weighed quantities of pure indigo from wool, low results are obtained. The largest percentage error was obtained with nitrobenzene; naphthalene came next, then aniline, and lastly phenol. From this it appears that the chemical properties of the respective solvents, as well as their boiling points, play an important rôle in the quantitative extraction of the indigo. None of the above solvents is as suitable as acetic acid.

The extraction of indigo from dyed materials by solvents, followed by sulphonation and estimation of the indigo by examining the colour of the solution tintometrically (in place of titration with permanganate) was also found unsatisfactory, concordant tintometric or colorimetric readings being very difficult to obtain.

II. New Methods of Analysis.—In the hope of finding a solvent capable of effecting a more rapid extraction of the indigo from the dyed material, whilst at the same time leaving unaffected any topping or bottoming colour which might be present, experiments have been made by Green, Gardner, Lloyd and Frank (*loc. cit.*) with numerous organic liquids. The following are the results obtained:

(*a*) Pyridine (pure or commercial) is a valuable solvent for the extraction of indigo. It removes the indigo quantitatively and more rapidly than does acetic acid, leaving the wool in better condition and with less loss of wool substance. In a number of cases in which the topping or bottoming colours are removed by acetic acid, they are but little affected by pyridine.

(*b*) Piperidine extracts indigo quantitatively, and has practically the same properties as pyridine, but is much more expensive.

(*c*) Anisole also extracts indigo quantitatively, but lacks sufficient solvent power for practical use.

(*d*) Epichlorhydrin dissolves indigo slowly, giving a clear blue solution. The extraction, though slow, is quantitative.

(*e*) Dichlorhydrin dissolves indigo, giving a green solution. The indigo may be completely removed from dyed fabrics, but cannot be estimated by means of this solvent, as some decomposition takes place.

(*f*) Formic acid, 90%, extracts indigo more readily than does glacial acetic acid, but attacks the wool to a greater extent.

(*g*) Indigo is removed from the fibre, though not very readily, by chloroacetic ether; more slowly by amyl alcohol, amyl acetate, cumene, and perchlorethylene.

(*h*) Benzaldehyde removes indigo from the fibre very rapidly; at the same time combining with the indigo to form a soluble yellow compound. Many topping or bottoming colours, which are stripped from the fibre both by acetic acid and by pyridine, are but little affected if the indigo is removed by benzaldehyde. It is therefore useless for the estimation of indigo, but is of value for the rapid qualitative testing of a dyed cloth.

(*i*) Cresol has long been known as a good solvent for indigo, but it attacks the wool too seriously to be used at its boiling point. It has, however, proved a most satisfactory agent when diluted with about 25% of a neutral hydrocarbon, such as "solvent naphtha" or "turpentine substitute," so as to give a liquid which will extract in

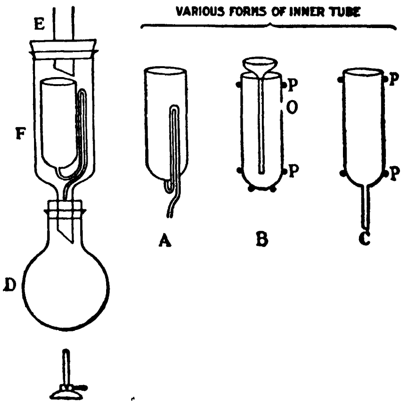


FIG. 5.

an ordinary Soxhlet extractor at a temperature of from 100° to 110°. By this means the indigo can be removed completely in nearly all cases without disturbing the concomitant dyestuffs, which can then be examined or quantitatively estimated.

Of the solvents experimented with, only glacial acetic acid, pyridine, benzaldehyde, and the cresol mixture appear to be capable of practical employment. The special use of each of these will be referred to later.

Extraction Apparatus.—In the ordinary Soxhlet extractor the solvent is much below its boiling point when it comes into contact with the fibre.

If means are taken to effect the extraction at the actual boiling point of the solvent, the time for extraction is greatly decreased.

Thus, when using glacial acetic acid or pyridine as solvents, the thickest materials can be completely extracted in 4 hours at most, whilst ordinary materials do not require more than 1½ to 2 hours. Extraction at the boiling point is effected by employing one of the forms of extractor which have been devised by L. L. Lloyd for this purpose (see Fig. 5, forms *A*, *B*, and *C*, on page 375).

If the form *A* is used the weighed cloth is placed loosely in the siphon tube, which is surrounded by an outer tube *F* through which the vapour passes. If the cloth is packed too tightly, indigo separates in a crystalline form on the inner surface of the tube, and is only slowly redissolved. The solvent is condensed by an air or water condenser *E*, and flows into the siphon tube, from which it is intermittently siphoned over into the distilling flask *D*. The extraction is continued until a blue extract is no longer obtained. To detect whether the whole of the indigo has been extracted, the Bunsen flame is regulated so that the liquid in the extraction tube just fails to siphon over, and the solvent is kept in contact with the material for about 10 minutes. If the extraction is complete, the liquid will not be coloured. In the case of material which easily separates short fibres, it is preferable to pack it loosely in an inner tube drawn out at the end and provided with glass points to support it in the siphon tube. Some crushed quarts is placed in the latter to act as filter, and to prevent short fibres from closing up the fine opening in the tube.

In form *B*, the cloth is placed loosely in the extraction tube and the condensed solvent directed to the bottom by means of a long, drawn-out funnel tube, the solvent then overflowing from the side opening (*o*) in the extraction tube.

The simpler form of apparatus *C* is found to give good results in many cases, and is easy to make. The extraction tube is drawn out at the end, some glass points (*P*) being fused on to the outer surface so as to furnish a passage round it for the vapour of the solvent. A little white wool, cotton wool, or crushed quartz is placed in the tube, as a filtering and regulating medium, and also to retain short fibres. The orifice and packing should be so adjusted as to prevent the condensed solvent from running through too quickly thus insuring the accumulation of a head of liquid above the cloth. This may be regulated by the flame and by the packing of the cloth and filtering medium.

Form *A* is the most satisfactory for most kinds of fabrics, but *B* is to be preferred for loosely woven and open cloths. Form *C* is suitable for materials easily separated into fibres, the threads, if necessary, being held in position by covering with a small filter plate. In the extraction of heavily-dyed, thick, felted cloth, it is recommended to cut the material into small pieces or strips and to commence the extraction in the apparatus *C*, afterwards placing the tube containing the partially extracted material in the siphon tube *A*.

The weight of the cloth to be taken for gravimetric estimations varies from 3 to 15 grm., viz., sufficient to give from 0.03 to 0.10 grm. of indigo.

Acetic Acid Extraction.—The extraction of the indigo is accompanied by a certain loss of weight in the wool, and the extracted wool substance is not soluble in water but is precipitated with the indigo, which accounts for the incorrect results obtained when such precipitated indigo is sulphonated and estimated by titration.

The following method should be adopted: The indigo is extracted at the boiling point in one of the forms of apparatus already described, using 3 to 15 grm. of material according to the percentage of indigo present. From 50 to 70 c.c. of glacial acetic acid are employed, and with the extractor *A* or *B* an additional quantity sufficient to fill the extraction tube and cause it to siphon or overflow. At the end of the extraction the tube is left filled with the solvent. If the material is easily extracted, the boiling-flask may be heated over wire gauze, but when the time of extraction exceeds 2 hours the flask should be heated in an oil-bath. Under these conditions the amount of decomposition is not sufficient to affect the accuracy of the process for technical purposes.

The extract is allowed to stand until cold and is then filtered through a weighed glass tube containing quartz or glass wool, or upon a Gooch crucible. The indigo is washed twice with 10 c.c. of cold glacial acetic acid, then with 20 c.c. of boiling dilute acetic acid (30% by volume), and afterwards with water. To remove wool substance and secondary colouring matters, the indigo is then well washed with boiling dilute sulphuric acid (20% by volume), the acid is removed by washing with water, and the indigo is then well washed with boiling ammonia (1:3) or with boiling 10% sodium hydroxide until the filtrate is no longer coloured. The alkali is removed by washing with boiling water, then with a small quantity

of acetic acid, again with boiling water, and finally with about 20 c.c. of alcohol. The filter is now dried at 110° and weighed.

Alternatively the extracted and purified indigo is sulphonated at 70°–75° with 15–20 c.c. of pure conc. sulphuric acid for three-quarters of an hour. After cooling, it is poured into water, made up to 500 c.c., and titrated with N/50 permanganate, using 100 c.c., diluted with 200 c.c. of water. The indigo content is obtained from the factor 1 c.c. N/50 permanganate = 0.00146 grm. indigo.

Extraction with Pyridine.—The powerful solvent action of pyridine upon indigo renders this liquid particularly suitable for extracting indigo from the fibre, and superior in many respects to acetic acid. The wool substance is not dissolved to the same extent by pyridine (1.8%) as by acetic acid (5.5 to 6%) and sodium hydroxide completely removes the impurity from the precipitate.

Iron lakes of tannin materials are fairly easily decomposed by pyridine, and when the wool has been heavily loaded with iron the precipitated indigo will require a more protracted washing with acid and alkali.

The use of pyridine has especial advantages in the analysis of thick, felted, heavily milled or hard spun twill materials, which are very troublesome and difficult to extract completely by means of acetic acid. It also has the advantage of leaving the wool in a better condition than does acetic acid.

The following procedure should be adopted: The extraction tube is charged with from 3 to 15 grm. of material (viz., sufficient to give about 0.05 grm. of indigo) enclosed in thin wire gauze. 100 c.c. of commercial pyridine (b. p. 110°–127°) are put into the boiling flask, which is heated over wire gauze or upon an air-bath. Either a water condenser, or simply a long air condenser, is employed. The extraction is continued until the pyridine runs through quite colourless, which usually requires from 2 to 3 hours. The extract is then distilled down to about 20–30 c.c., the pyridine recovered being kept for future extractions. The extraction flask is then set aside to cool, when the greater part of the indigo separates in well-formed bronzy crystals. To complete the precipitation of the indigo, 150 c.c. of 50% alcohol are added, and after heating to boiling, the liquid is filtered through a Gooch crucible prepared with filter paper or asbestos.

Before weighing the filter for use, it is washed with exactly the same liquids as are to be used for washing the precipitated indigo and

then dried at 110°. The filtration through a Gooch crucible is very rapid, taking less than 2 minutes. The precipitate is washed on the filter successively with hot 50% alcohol, hot 2% sodium hydroxide solution, hot dilute hydrochloric acid (3:100), hot water, alcohol, and finally ether. The crucible is then dried at 110° and weighed. The appearance of the indigo precipitate is a guide to its purity. It should form a bronzy crystalline powder, which, when analysed, tests 100%. A dull appearance shows the presence of impurities. In order to shorten the operation it is advisable after about ¾ of an hour's extraction to remove and repack the material in the tube, placing what was formerly inside in the outside position. Heavy materials should be cut into fine strips and also repacked after some time. If it is desired to estimate the indigo by titration instead of by direct weighing, the precipitate is collected on a Gooch crucible, the bottom of which is covered with a little asbestos. After washing with acid and alkali as above, and drying for a short time, the crucible is placed in a small beaker containing 15 to 20 c.c. of pure concentrated sulphuric acid, and the indigo is sulphonated by heating in an oven to 70°–80° for 45 minutes. The solution is then titrated with N/50 permanganate, in the usual way.

The following table (Lloyd) gives the limits of accuracy in the various methods of estimating indigo on dyed material.

		Possible error (percentage upon total indigo)
I.	Rawson's hydrosulphite method	0 to −13
II.	Brylinski's method	+30.0 to +44.7
III.	Acetic acid extraction followed by after-treatment with sodium hydroxide	−1.7 to −2.8
IV.	Möhlau and Zimmermann's method	−3.8 to −11.0
V.	Acetic acid extraction followed by washing with 20% sulphuric acid and sodium hydroxide or ammonia	−0.5 to +3.1
VI.	Preceding method when other dyestuffs are present	−1.3 to +3.1
VII.	Pyridine extraction without after-treatment of precipitate	+8.7 to +30.0
VIII.	Pyridine extraction followed by washing with 20% sulphuric acid and sodium hydroxide or ammonia	−2.2 to −4.0
IX.	Preceding method when other dyestuffs are present	−3.4 to +1.5

Analysis of Commercial Indigo-dyed Materials by the Pyridine and Acetic Acid Methods.—Both the acetic acid and pyridine methods are capable of giving accurate results under the conditions laid down.

In order to subject these methods of analysis to a rigid test, and at the same time to effect a comparison between them, analyses were carried out upon a large number of materials dyed under practical conditions, the results of which are given in the tables which follow. The first series represents a range of pure indigo shades, from a light blue to a very dark navy. The second series consists of a medium shade of indigo-dyed cloth which was afterwards topped with a variety of acid and mordant colouring matters. In the third series the wool was bottomed with various colouring matters and afterwards dyed in the indigo vat. The colouring matters selected as topping or bottoming colours were those most likely to be employed in practice. The fourth series consists of a variety of commercial indigo and navy blue cloths of different makes. The fifth series contains a number of official and Government cloths. In series six the same dystuffs are employed both as bottoming and topping colours, in order to ascertain whether the total tintometric value of the mixed shade is thereby affected.

The analyses were made gravimetrically with pyridine and both gravimetrically and volumetrically with acetic acid.

SERIES I. (PURE INDIGOS)

Reference number	Material dyed with	Percentage indigo found		
		Acetic acid or other solvent[1] by gravimetric estimation (Lloyd)	Acetic acid by titration with $KMnO_4$ (Frank)	Pyridine by gravimetric estimation (Frank)
		%	%	%
1	Pure indigo.....	0.42	0.54	0.44
2	Pure indigo......	0.51	0.66	0.54
3	Pure indigo......	0.71	0.75	0.64
4	Pure indigo......	0.85	0.92	0.80
5	Pure indigo......	1.14	1.08	1.05
6	Pure indigo......	1.30	1.29	1.25
7	Pure indigo......	1.46	1.44	1.33
8	Pure indigo......	1.68	1.71	1.70
9	Pure indigo......	2.13	2.20	2.27
10	Pure indigo......	2.43	2.50	2.50
59	Pure indigo......	2.23	2.10	2.10
60	Pure indigo......	2.47	2.41	2.35
61	Pure indigo......	2.72	2.95	2.71
62	Pure indigo......	3.69	3.54	3.42
63	Pure indigo......	4.57	4.43	4.42

[1] Some of these figures are the average of several obtained with acetic acid, pyridine, and piperidine.

SERIES II. (COMPOSITE DYES)

Reference number	Material dyed with	Percentage of indigo found: Acetic acid or other solvent[1] by gravimetric estimation (Lloyd)	Acetic acid by titration with $KMnO_4$ (Frank)	Pyridine by gravimetric estimation (Frank)
		%	%	%
13	Standard Indigo, bottomed with 2 % chrome alone..	1.68	1.79	1.73
	Standard Indigo, topped with:			
14	2 % dichromate and 2 % Sulphon Cyanine 5R extra.	1.69	1.72	1.72
15	2 % dichromate and 1½ % Brilliant Aliz. Blue R pdr..	1.71	1.74	1.67
16	1½ % Topping Violet RTN (B.A.S.F.).......	1.67	1 76	1.66
17	2 % Indocyanine 2R (Berlin. Co.)	1.68	...	1.72
18	1½ % Erio Fast Purple A (Geigy)..............	1.69	1.77	1.69
19	2 % Fast Acid Violet R (M. L. & B.)	1.71	1.75	1.72
20	1¼ % dichromate and 2 % Chrome Blue A (B.A.-S.F.).......	1.64	1.73	1.70
21	1¼ % dichromate and 2 % Palatine Chrome Blue B	1.59	1.78	1.77
22	2 % dichromate and 2 % Hæmatine crystals ..	1.70	1.68	1.70
23	1¼ % dichromate and 2 % Eriochrome Azurol B..	1.65	1.72	1.68
24	1¼ % dichromate and 2 % Omega Chrome Cyanine B..	1 68	1.67	1.62
25	2 % dichromate and 1¼ % Alizarin Blue B..	1 71	1.68	1.69
26	2 % dichromate and 8 % Alizarin Blue GW double..	1 67	. ..	1.65
27	2 % dichromate and 2 % Sulphon Dark Blue 2B.	1.72	1.69	1.72
28	2 % dichromate and 2 % Wool Fast Blue BL (By).	1.67	1.74	1.62
29	2 % dichromate and 2 % Sulphon Cyanine GR extra	1.69	1.69	1.65
30	2 % dichromate and 1½ % Indochromine 2R conc	1 73	1.69	1.69
31	2 % dichromate and 20 % Cudbear	1.70	1.65	1.65
39	2 % dichromate and 7 % Gallein paste	1.67	1.74	1.66
40	2 % dichromate and 7 % Gallocyanin paste.....	1.74	1.73	1.62
41	2 % dichromate and 5 % Alizarin Cyanin 3R double paste	1 69	1.73	1.60
42	2 % dichromate and 2 % Lanacyl Violet B (Cass.).	1 65	1.76	1 69
43	2 % dichromate and 2 % Soluble Blue	1 69	1.73	1.68
44	2 % dichromate and 2 % Acid Chrome Blue 2R (By.)	1.65	1.75	1.65
45	2 % dichromate and 2 % Eriochrome Blue BR (Gy.)...........	1 67	1.74	1.62
46	1 % Acid Violet 4BRS (Sandoz)......	1 70	1.76	1.72
47	1½ % Omega Light Violet R (Sandoz)......	1.72	1.75	1.72
50	Myrabolans and "nitrate of iron"..	1 71	1.73	1.66
55	3 % dichromate and 3½ % Fustic...	1.73	1 72	1 64
56	2 % Picric Acid	1.70	1.76	1 68
57	2 % dichromate and Logwood.	1.72	1 70	1.62
58	Myrabolans and ferrous sulphate...	1 72	1.65	1.63
83	Indigo extract..	1.66	....	1.66
	Standard Indigo filled with:			
85	Starch.	1.62	1.60	1.62
86	Magnesium chloride . .	1.61	..... .	1 62

[1]Some of these figures are the average of several obtained with acetic acid, pyridine, and piperidine.

SERIES III. (BOTTOMED BLUES)

Reference number	Other dyes topped with indigo		Percentage of indigo found: Acetic acid or other solvent[1] by gravimetric estimation (Lloyd)	Percentage of indigo found: Acetic acid by titration with $KMnO_4$ (Frank)	Percentage of indigo found: Pyridine by gravimetric estimation (Frank)
	Bottomed with:	All dyed together in same indigo vat.	%	%	%
32	1% Azo Fuchsine G		2.20	2.37	2.14
33	2% dichromate and 8% Cudbear		2.16	2.02	1.97
34	2% dichromate and 1% Aliz. Red IWS(M)		2.09	2.07	1.94
35	2% dichromate and 20% Sanderswood		2.13	2.00	1.98
36	2% dichromate and 30% Camwood		2.23	2.05	2.02
37	2% dichromate and 20% Barwood		2.14	2.05	1.96
38	1% dichromate and 1% Omega Chrome Red B.		2.20	2.13	2.08

[1]Some of these figures are the average of several obtained with acetic acid, pyridine, and piperidine.

SERIES IV. (BLUES ON VARIOUS MATERIALS)

Reference number	Description		Percentage of indigo found: Acetic acid or other solvent[1] by gravimetric estimation (Lloyd)	Percentage of indigo found: Acetic acid by titration (Frank)	Percentage of indigo found: Pyridine by gravimetric estimation (Frank)
			%	%	%
64	Pure indigo on 2-ply worsted	Dyed together in same vat.	1.64	1.65	1.58
65	Pure indigo on fine woollen		1.98	1.97	1.89
66	Pure indigo on fine merino		2.30	2.40	2.28
67	Pure indigo on coarse worsted		1.61	1.78	1.54
68	Lighter shade of No. 64	Dyed together in same vat.	0.51	0.49	0.48
69	Lighter shade of No. 65		0.70	0.54	0.55
70	Lighter shade of No. 66		0.84	0.73	0.75
71	Lighter shade of No. 67		0.69	0.54	0.54
84	Indigo on cotton warp material		1.72	....	1.63
51	A commercial indigo		0.98	0.71	0.71
52	Another commercial indigo		1.03	0.70	0.65
53	No. 1 Navy blue		3.86	3.76	3.80
54	"Pure" indigo on worsted cloth (red bottom)		3.44	....	3.41
12	No. 2 navy blue on grey serge		2 38	....	2.32

[1] Some of these figures are the average of several obtained with acetic acid, pyridine, and piperidine.

SERIES V. (OFFICIAL AND GOVERNMENT STANDARDS)

Reference number	Description	Percentage of indigo found by extraction with pyridine (by gravimetric estimation) (Lloyd),[1]	(Frank),
		%	%
72	No. 1 customs	2.52	2.35
73	No. 2 customs worsted	2.59	2.54
74	No. 3 blue	4.18	4.18
75	(Old) Pantaloon cloth	3.35	3.23
76	Post-office pilot	3.12	3.22
77	4A blue	3.25	3.0
78	Box cloth	4.20	4.23
79	4B blue	2.60	2.54
80	(Old) Metropolitan Police blue	2.90	2.82
81	No. 2 artillery	3.04	3.11
82	Navy tartan	3.03	2.91
87	Metropolitan Police, blue greatcoat	2.46	2.50

[1] In some cases analyses were made with piperidine as well as with pyridine.

SERIES VI. (EFFECT OF TOPPING OR BOTTOMING ON TOTAL SHADE)

Reference number	Description		Percentage of indigo found: Acetic acid or other solvent[1] by gravimetric estimation (Lloyd)	Pyridine by gravimetric estimation (Frank)
			%	%
109	White cloth dyed with Indigo	All dyed at same time in indigo vat.	1.36	1.34
	Bottomed with			
110	34 % dichromate and 1 % Eriochrome Red BR		1.54	1.56
111	1 % Eriochrome Red BR without chrome		1.53	1.54
112	2 % dichromate and 1 % Alizarin Red IWS		1.32	1.34
113	Previously chromed wool (2 % chrome) dyed with Indigo.		1.37	1.47
	Indigo, No. 109, topped with			
114	1 % Eriochrome Red BR and 34 % dichromate		1.35	1.36
115	1 % Eriochrome Red BR without dichromate		1.33	1.36
116	1 % Alizarin Red IWS and 2 % dichromate		1.26	1.41
	Indigo, No. 113, topped with			
117	1 % Eriochrome Red BR and 34 % dichromate		1.37	1.48
118	1 % Eriochrome Red BR without dichromate		1.36	1.48
119	1 % Alizarin Red IWS and 2 % dichromate		1.24	1.46

[1] Some of these figures are the average of several experiments wiih different solvents.

III. Estimation of Percentage Colour Effect Due to Indigo.—It is obvious that the simple statement of the percentage of indigo upon a cloth, as found by analysis, does not afford to anybody but an expert an idea of the quality of the dye. What is required by manufac-

turers, merchants, and the public is a means of knowing what *proportion* of the total depth of colour is due to the indigo present. The solution of this problem is a difficult one, as besides the difficulty of finding an instrument capable of accurately measuring depth of colour on fabrics, the dyestuffs accompanying the indigo are not necessarily blue, but are frequently violet or red. The most satisfactory solution has been found by employing the Lovibond tintometer (see Vol. VI) as the colour-measuring instrument. Since the relative proportions of red, yellow, and blue will vary in different

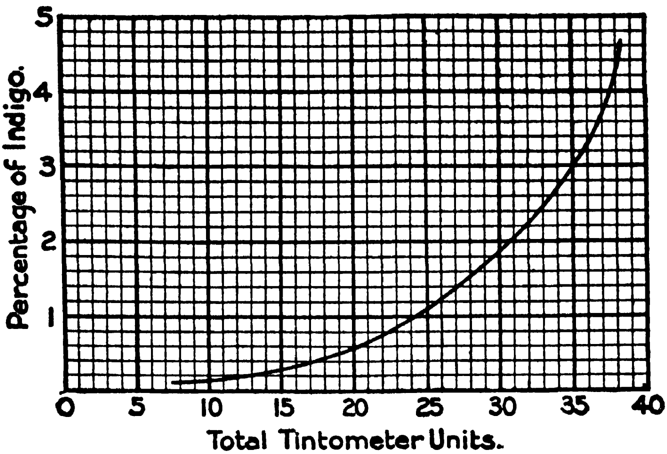


FIG. 6.

shades, the measure of depth must be taken as the total number of colour units obtained by adding together the units of red, yellow, and blue, given by the glasses required to match the pattern. By applying this method to the series of shades of pure indigo, dyed on white wool, of which the analyses are given in the table Series I, it has been found that the depths of shade thus expressed lie upon a regular curve (see Fig. 6). There is thus a definite relation between the percentage weight of indigo on the material and the tintometric reading. The tintometer readings from which this curve is constructed are given in the following table. These readings were obtained in a north light between 10 a. m. and 12 a.m. on February 18, 1914, a bright morning with light blue sky and white clouds:

Reference number	Percentage of indigo present (by analysis). (Mean result)	Tintometer measurements			
		Red	Yellow	Blue	Total units
1	0.45	4.0	2.6	10.9	17.5
2	0.55	4.9	2.9	11.4	19.2
3	0.70	6.1	3.4	12.1	21.6
4	0.85	7.1	3.5	12.7	23.3
5	1.05	8.4	3.5	12.96	24.86
6	1.25	9.0	4.0	13.0	26.0
7	1.40	9.6	4.3	13.1	27.0
8	1.70	10.8	4.7	13.5	29.2
9	2.20	11.7	5.9	13.6	31.2
10	2.50	12.2	6.6	13.7	32 5
61	2.71	12.5	8.3	13.9	34.7
62	3.55	13.2	9.4	14.0	36.6
63	4.45	13.7	9.8	14.5	38.0

By means of such a curve it is possible to estimate from the tintometric readings of a given cloth, the percentage of indigo it contains if dyed with indigo only; or when dyed with other colours in addition, the percentage of indigo which would be required to give a shade of corresponding depth if indigo alone was used. In the latter case when the actual percentage of pure indigo on the cloth, as estimated by analysis, is known, it is possible to express the colour effect due to indigo (obtained from the curve) as a percentage of the total colour, measured in terms of the amount of indigo which would be required to give the total depth of shade of the pattern if indigo alone was used. This ratio, termed the "percentage colour effect due to indigo," is given by the expression:

$$x = \frac{A \times 100}{C}$$

in which A = the percentage of indigo found by analysis and C = percentage of indigo given by the curve corresponding to the total tintometer units of the pattern.

Experiments made with this method have shown that for blues of light and medium depth (up to, say, 2.5% of indigo) results of sufficient exactitude for commercial purposes can be obtained, but that for heavy shades which approach black the tintometer is incapable of measuring colour depth with sufficient accuracy, since with dark shades a relatively large increase in the percentage of indigo produces only a small increase in the tintometric reading.

Unless a standard source of light is used throughout it is recommended that in applying this method the curve given above

should only be used as an approximation and should be corrected at the time of use by taking tintometer readings of a series of four or five pure indigo shades kept as standards, in which the percentages of indigo have been accurately estimated by analysis. Having also obtained by analysis the percentage of indigo in the material under examination, the value for the "percentage colour effect due to indigo" can then be deduced from the corrected curve. For example, a sample of cloth dyed with indigo and topped with other dyestuffs was found to contain 1.5% of indigo by analysis, and gives a total reading of 32.5 colour units on Lovibond's tintometer. From the curve it was seen that a shade dyed with pure indigo to give a reading of 32.5 colour units had to contain 2.4% of indigo. Therefore, the "percentage colour effect due to indigo" on the pattern was:

$$\frac{1.5 \times 100}{2.4} = 62.0$$

For the investigation of heavy shades, with which the above method of deducing the "percentage colour effect due to indigo" is unreliable, another method is available which appears to be capable of giving satisfactory results with all depths of shade. This consists in entirely removing the indigo from the pattern by extraction with a suitable solvent and measuring the depth of the residual colour (dyestuff used for topping or bottoming) in total colour units by the Lovibond tintometer. The indigo equivalent of this residual colour ($= B$) is then found from the curve, and knowing the actual percentage of indigo present on the cloth, as estimated by gravimetric analysis ($= A$), the "percentage colour effect due to indigo" is given by the equation:

$$x = \frac{100A}{A + B}$$

To make use of the second method it is necessary to be able to strip the indigo completely from a compound shade and to leave the topping or bottoming colour practically unaltered. A small *change* of shade of the latter does not matter, provided the *depth* is not affected. In a large number of cases the stripping may be satisfactorily done by employing either boiling glacial acetic acid or boiling pyridine as the stripping agent; for whereas many dyes are removed from wool by one or the other of these solvents, comparatively few are stripped by both, those which are dissolved by the one being usually not affected by the other. In using these

solvents care should be taken that they are anhydrous, as a small percentage of water increases their solvent action on dyestuffs other than indigo. The acetic acid should therefore be frozen and

Name of colouring matter	Acetic acid	Pyri-dine	Benzal-dehyde	Cresol mixture
Sulphoncyanine 5R extra and GR (By.)	−	−	+	+
Brilliant Alizarin Blue R (By.)	+−	+−	+	+
Topping Violet RTN (B.A.S.F.)	+	−	+	+
Indocyanine 2R (Ber.)	+	+	+	+
Erio Fast Purple A (Gy.)	+	−	+	+
Fast Acid Violet R (M.L. & B.)	+−	+−	+	+
Chrome Blue A (B.A.S.F.)	+	+	+	+
Palatine Chrome Blue 2B (B.A.S.F.)	+	+	+	+
Hæmatine crystals	−	+	+	+
Eriochrome Azurol B (Gy.)	−	+	+	+
Omega Chrome Cyanine B (Sz.)	+	+	+	+
Alizarin Blue Black B (By.)	+−	+	+	+
Alizarin Blue SW (B.A.S.F.)	+−	+	+	+
Sulphon Dark Blue 2B	−	−	+	+
Wool Fast Blue BL (By.)	+*	−	+	+
Indochromine 2R conc. (S.)	+*	+	+	+
Cudbear on dichromate	−	−	+−	+
Azofuchsine	−	−	+	+
Alizarin Red 1WS (M.L. & B.)	+−	+	+	+
Sanderswood and dichromate	−	+−	+−	+−
Camwood and dichromate	−	+−	+−	+−
Barwood and dichromate	−	+−	+−	+−
Omega Chrome Red B	+	−	+	+
Gallein paste	−	+	+	+
Gallocyanin	−	+	+−	+
Alizarin Cyanine 3R	−	+	+	+
Lanacyl Violet B (Cass.)	+	−	+	+
Soluble Blue	−	−	−	+−
Acid Chrome Blue 2R (By.)	+*	+−	+	+
Eriochrome Blue BR (Gy.)	+	−	+	+
Acid Violet 4BRS (S.)	+	−	+	+
Omega Light Violet R (S.)	+	−	+	+
Myrabolans and iron	−	+	−	+
Fustic and dichromate	−	+	+	+
Picric acid	−	−	+	+−
Logwood and dichromate	−	+	+	+
Indigo extract	+	−	+	+
Eriochrome Red BR (Gy.)	+	−	+	+

the separated crystals remelted, whilst the pyridine should be carefully dried over solid sodium hydroxide. For the same reason, the pattern to be extracted should be previously dried in a steam oven.

As there are a few colouring matters which are removed from the wool of both acetic acid and by pyridine, some other solvent is occasionally necessary. Benzaldehyde can be employed with good effect in many cases, but usually the best extracting agent is a mixture of 100 parts cresol (commercial cresylic acid, 97–98%) with 30 parts of "solvent naphtha" of boiling point 125°–140°.

The temperature should not exceed 110°, (preferably 100°–105°). The lower the temperature and the shorter the time used for the extraction of the indigo the less the danger of disturbing the concomitant dye-stuffs. Carried out with care this method of separation seems capable of almost universal application, and the only cases in which the original depth of the bottoming or topping colour was not obtained were with Soluble Blues, Picric Acid, and redwoods, in which there was strong evidence that the bottom dye had already been partly removed in the indigo vat.

The behaviour of the four selected solvents towards a variety of colouring matters dyed upon wool in conjunction with indigo is shown in the table on p. 387. The minus sign indicates that the colour is stripped, the plus sign that it is not affected at all or only slightly, and the sign (+ —) that it is partially removed. Those dyes marked by an asterisk are changed in shade to violet.

A number of official and Government cloths (Series V p. 383) gave the following results: In each case the tintometric readings of the stripped patterns were all made at the same time, and the units of total colour converted into indigo equivalents by reference to a curve which also was constructed at the time.

Number of sample	Indigo found by analysis ($=A$)		Indigo equivalent of residual colour ($=B$)		Percentage colour-effect due to indigo $\left(=\frac{100A}{A+B}\right)$	
	Frank	Lloyd	Frank	Lloyd	Frank	Lloyd
72	2.35	2.52	0.55	0.72	80	77
73	2.54	2.59	0.20	0.22	92	92
74	4.18	4.18	0.68	0.72	86	85
75	3.23	3.35	0.68	0.55	83	86
76	3.23	3.12	0.30	0.36	91	90
77	3.0	3.25	0.65	0.65	82	83
78	4.23	4.20	0.30	0.32	93	93
79	2.54	2.60	0.75	0.48	77	84
80	2.82	2.90	0.60	0.62	81	82
82	2.91	3.03	0.40	0.32	88	90

It appears from these results that the maximum error of the process is about 7% and with practice would certainly not exceed 5%.

Estimation of Indigo on Cotton.—For cotton, Knecht's process (*J. Soc. Dyers and Col.*, 1909, **25**, 135 and 160) should be used. About 4 grm. of the material are treated with 25 c.c. of 80% sulphuric acid at 35° to 40°, for about 10 minutes. This dissolves both the cotton and the indigo. The solution is diluted to about 120 c.c. with water and boiled for a few minutes. This precipitates the indigotin which is filtered off through a Gooch crucible lined with asbestos or silica. The indigotin is washed, dried, sulphonated and estimated in the usual way.

LOGWOOD

Logwood is the product of a large leguminous and rapidly growing tree, *Hæmatoxylon campechianum.* It was imported originally from the Bay of Campeachy, but the supply now chiefly comes from Jamaica and Honduras. It is also exported from San Domingo, Cuba, Haiti, etc., but the best quality now comes from Yucatan.

When first cut down the wood has a yellowish-brown colour, but on exposure to the atmosphere it gradually develops superficially a rich brownish-red colour. It is imported in the form of rough logs, which, before use, are reduced to small chips or rasped to powder, these products being distinguished respectively as "chipped," and "rasped" or "ground" logwood.

Logwood extracts are obtained by treating the chipped wood with water in a vacuum pan and concentrating the liquid under reduced pressure. The extract amounts to about 15% of the weight of the wood.

Colouring Matter of Logwood.—The freshly chipped wood contains from 5–10% of a colourless compound, *hæmatoxylin*, $C_{16}H_{14}O_6$, which when pure forms white prismatic crystals. It was first isolated by Chevreul. It is slightly soluble in cold and easily in hot water, alcohol, ether, or CS_2. It is dextrorotatory. It readily reduces silver salts and gives a rose-coloured coloration with alum. It is closely allied to *brazilin* the corresponding substance existing in the "soluble redwoods". On fusing with alkali hydroxide it yields pyrogallic acid, $C_6H_3(OH)_3$. Much research has been done on the constitution of hæmatoxylin by W. H. Perkin, V. Kostanecki, Hirzig and others, the references being too voluminous to be inserted here. The whole controversy is summarised in *The Natural Organic Colouring Matters* by A. G. Perkin and A. E. Everest, p. 364 *et seq.* Hæma-

toxylin has no dyeing power. It has feebly acid properties and is not a glucoside. In the presence of alkali, it rapidly absorbs oxygen and is converted into the true colouring matter, *hæmatein*. This change is brought about more or less completely during the so-called "ageing" of logwood, a process which consists in subjecting the moistened ground wood to atmospheric oxidation by exposing it in heaps in a warm room. Hæmatoxylin is thus the *colouring principle* of logwood from which the true *colouring matter*, hæmatein, is formed by oxidation.

$$\underset{\textit{Hæmatoxylin}}{C_{16}H_{14}O_6} + O = \underset{\textit{Hæmatein}}{C_{16}H_{12}O_6} + H_2O.$$

Hæmatein, when pure, forms brownish-red crystals. It is almost insoluble in cold water, but is soluble in hot water or alcohol. It behaves as a weak acid and forms soluble salts with sodium, potassium, and ammonium, which possess a beautiful purple colour. In conjunction with the heavy metals it forms strongly coloured insoluble salts, or colour-lakes, upon the formation of which the value of logwood as a dyestuff depends. The iron-lake is black; the chromium-lake, blue-black; the copper-lake, greenish-black, and the aluminium-lake, purplish-blue. Hæmatein is somewhat easily attacked by oxidising agents with formation of brown worthless products. It is decomposed by hot sulphuric acid, but is soluble unchanged in cold conc. sulphuric acid with a brownish-red colour. In "overaged" or "burnt" logwood the hæmatein has been more or less destroyed by oxidation. A similar defect is brought about by "overchromed" wool, in which case the excess of potassium dichromate oxidises and destroys the colouring matter.

The colour-producing substance of logwood may thus exist in 3 forms, viz., as hæmatoxylin, the colouring principle, as hæmatein, the colouring matter, and as the worthless brown overoxidation product.

Logwood Extracts.—These are now used more largely than the rasped or ground wood, being manufactured in large quantities not only in this country but in the neighborhood of the logwood plantations. The extract is prepared from the unaged wood by extraction with pure superheated water, the extract being concentrated in vacuum pans. The extracts are sold usually as pasty liquids of 51° Tw. ("logwood extract") or in the solid form ("solid logwood extract"). The extract, as first formed, contains essentially hæmatoxylin, but many forms of oxidised extract in which the colouring matter is chiefly present in the form of hæmatein are now on the

market. These are sold under such names as "hæmatein crystals," "oxidised logwood extract," "logwood extract for wool," etc.

The unoxidised extracts are generally used in cotton dyeing, the oxidised extracts being employed for wood and silk.

In its commercial form logwood extract may be stored for some time without deterioration, but when diluted it somewhat rapidly ferments with destruction of the colouring matter. Logwood is chiefly employed for dyeing blacks, or as the darkening constituent in browns, olives, greys, etc. On cotton and silk it is used in conjunction with iron mordants, on wool usually with potassium or sodium dichromate as mordant, though iron blacks on wool are not infrequent.

Certain "direct blacks" for wool were much employed a few years ago. These were sold in the form of pastes or dry powders containing logwood extract, ferrous sulphate, and oxalic acid; the latter acting as a solvent for the insoluble hæmatein-iron lake.

Bastard Logwood.—Trees of this variety are found in logwood plantations and greatly resemble logwood in appearance. The wood does not contain hæmatoxylin but a yellowish-green pigment of no value. If a sample of rasped wood contain any bastard wood, on sprinkling on filter paper moistened with sodium hydroxide solution the true logwood particles produce violet spots and the particles of bastard wood yellowish pink spots.

South African Logwood.—This species of *hœmatoxylon* (*H. Africanum*) has recently been recognised. It does not contain hæmatoxylin but a colouring substance more closely related to brazilin. It is of no commercial value. (*The Natural Organic Colouring Matters* Perkin and Everest p. 382.)

Valuation of Logwood and Logwood Extract.—Since logwood and logwood extract contain a large amount of coloured soluble substances other than colouring matters, it is obviously impossible to estimate the amount of the latter by any direct colorimetric process. Further, hæmatoxylin is a colourless substance, though it is readily converted into hæmatein, and this change frequently takes place during the actual dyeing process. Any exact estimation of the value of a sample of logwood should thus involve an estimation of (*a*) hæmatoxylin, (*b*) hæmatein, and (*c*) impurities; but no satisfactory method of doing this has been devised.

The most reliable means of estimating the value of samples of logwood or logwood extract is by means of comparative dye trials

carried out under the exact conditions under which the dye is to be practically employed. It is essential that strict attention be paid to this point since the practical value of any sample depends largely on the process used in applying it.

For instance, in the case of two samples containing the same amount of colouring matter, but in the one case chiefly in the form of hæmatoxylin and in the other of hæmatein, the former would be most valuable in cotton dyeing, whereas the latter would be the best for use in wool dyeing; though by slight modifications of process either could be used for either purpose. The nature of the impurities may also have an important influence upon the value for a particular purpose. For example, a logwood extract to which 20% of chestnut extract has been added would be unsuitable for use in wool dyeing, since boiling with tannin matters tends to give wool a harsh feel. For use in the black dyeing of cotton this extract might, however, be quite satisfactory.

In carrying out dyeing experiments, amounts should be used which will produce greys only, in order that small differences may be recognisable.

In testing samples for use in wool dyeing, the yarn or cloth in 10 grm. lots is mordanted with 3% of potassium dichromate, raising to the boiling point in about half an hour and boiling for half an hour. The necessary number of pieces may conveniently be mordanted all in the same vessel in order to ensure equal treatment. Then, after washing, the mordanted patterns are separately dyed with about 10% of ground logwood or 2–3% of logwood extract. Similar experiments should be carried out on wool mordanted with 3% potassium dichromate and 1% sulphuric acid (oxidising mordant) and with 3% potassium dichromate with 4% tartaric acid (reduced mordant). By comparing the 3 sets of dyed patterns some idea of the degree of oxidation, as well as of the amount, of colouring matter present will be arrived at.

Hæmatoxylin will produce a very pale colour on the reduced mordant, but will dye up fully on the oxidising mordant. Hæmatein will dye well on the reduced mordant, but will produce a dull colour on the oxidising mordant since some over-oxidation will take place.

Dyeings may also usefully be made on cotten cloth printed in stripes with various mordants. This is not an article of commerce but is prepared and used by calico printers for colour-testing purposes.

During the "ageing" process, or in the manufacture of the extract, logwood is sometimes treated with an alkali, such as lime water, with a view of giving it a fictitious appearance of strength. If so treated, the wood yields its colouring matter more readily to water, but over oxidation and deterioration also occur more readily.

Logwood extracts are frequently adulterated with molasses, dextrin, or tannin extracts. For the detection of these substances 2 grm. of the extract are dried at 100° and then extracted with absolute alcohol until the solution gives no further reaction for hæmatein with sodium aluminate. On extracting the residue with water, molasses and dextrin may be detected in the usual manner. Logwood extract may normally contain 0.5% of dextrose.

Tannin matter is also a normal constituent, but if added as an adulterant will be present in excessive amount and may be detected by the strong grey or black colour produced by a cold dilute solution of the extract upon cotton mordanted with ferric iron.

The amount of moisture in logwood and logwood extracts varies within wide limits, and should always be estimated. The presence of any considerable amount of inorganic matter such as salt, sodium sulphate, or chalk, points to adulteration.

The reactions of an aqueous decoction of logwood are due to the simultaneous presence of hæmatoxylin and hæmatein. Dilute acids turn the solution yellow, but with excess of a strong acid a red colour is produced. Hydrogen sulphide or sulphurous acid partially decolourises a solution of logwood, turning it yellow. Alaklies and ammonia produce first a red, then a violet, and ultimately a brown colour; while lime, baryta, and most of the hydroxides of the heavy metals produce blue precipitates. Stannous hydroxide behaves as a base and yields a violet lake, while stannic hydroxide reacts as an acid and turns a logwood solution red. Salts of iron yield a bluish-black coloration, a reaction which is employed for producing *ink*. Mercuric chloride yields an orange, tartar-emetic a carmine, and bismuth nitrate a fine violet precipitate with logwood solution. Alum gives at first a yellow coloration, which turns red after a time; while sodium aluminate yields an abundant bluish-violet precipitate, insoluble in excess of alkali. This test is so delicate and characteristic that by means of it logwood may frequently be detected in a mixed decoction with great facility. Another characteristic reaction of logwood is the black coloration it produces with a dilute solution

of dichromate. This develops slowly, and, on boiling, a black precipitate is produced.

Detection of Logwood on the Fibre.—In the absence of other colouring matters the detection of logwood on the fibre is not difficult. In the case of wool the ash obtained on ignition will probably contain chromium which may be detected by fusing the ash with potassium chlorate. If a bright yellow mass is obtained, it is dissolved in water and a few drops of acetic acid are added; a drop of lead acetate solution will then produce a bright yellow precipitate of lead chromate.

Logwood on cotton will yield on ignition an ash containing iron; the ash has a reddish-brown colour and its solution in hydrochloric acid gives a blue precipitate with potassium ferrocyanide.

The presence of chromium or iron is, of course, merely an indication, not a proof, of the presence of logwood.

If employed in conjunction with other colouring matters, such, *e. g.* as gallocyanin, the detection of logwood is not easy, but the following reactions of logwood will usually enable a judgment to be formed. On boiling with dilute (5%) hydrochloric acid a cherry-red solution is obtained, the fibre becoming purple or drab. On adding excess of alkali to the acid solution a deep violet colour is produced, the liquid gradually depositing a brown precipitate.

Concentrated hydrochloric acid produces a red spot on logwood-dyed material; if this spot is pressed against a piece of filter-paper it produces a red stain which turns blue if touched with a glass rod moistened with sodium aluminate.

All logwood dyes are readily bleached by hypochlorites.

The following procedure is recommended for distinguishing logwood in the presence of *alizarin blue*, *gallocyanin*, or *indigo*.[1]

The sample is treated in a porcelain dish with cold concentrated sulphuric acid. Indigo gives a blue solution which remains blue on dilution; Alizarin Blue, a violet-blue liquid which becomes violet-red on dilution; Gallocyanin gives a violet liquid which becomes redder on dilution; logwood gives a brownish-red solution which becomes yellow on dilution, and this yellow even in small amounts so greatly modifies the pinks due to Alizarin Blue and Gallocyanin that its detection is quite easy if comparison is made with similar solutions prepared from known dyestuffs.

With a mixture of indigo and logwood the sulphuric acid solution is green and remains green on dilution, but on passing several times through a filter the indigo is removed and the solution becomes yellow.

[1] Manual of Dyeing, Knecht, Rawson and Loewenthal, 2d Ed., 1910, p. 348.

NATURAL YELLOW COLOURING MATTERS

The following table includes the chief natural yellow colouring matters:

Commercial name	Source		Colouring principle	
	Botanical	Geographical	Name	Formula
Old Fustic; Yellow Brazil Wood.	Wood of *Morus tinctoria.*	West Indies; South America. India.	Moric acid. Moritannic acid or Maclurin.	$C_{15}H_{10}O_7$ $C_{13}H_{10}O_6$
Weld.	Leaves, etc., of *Reseda luteola.*	France, Britain, Belgium.	Luteolin.	$C_{15}H_{10}O_6$
Quercitron.	Bark of *Quercus nigra* or *Q. tinctoria.*	North and Central America.	Quercitrin Quercetin.	$C_{21}H_{20}O_{11}$ $C_{15}H_{10}O_7$
Turmeric.	Underground stem of *Curcuma tinctoria* or *longa* and *C. rotunda.*	East Indies; China, Barbadoes.	Curcumin.	$C_{21}H_{20}O_6$
Gamboge.	Gum resin from *Garcinia morella.*	Siam, Cochin China, Ceylon.	Gambogin.	$C_{30}H_{35}O_6$
Saffron.	Stigmata of flower of *Crocus sativus.*	Austria, Spain, France.	Crocin. Crocetin.	$C_{44}H_{70}O_{28}$ $C_{34}H_{46}O_9$
Young Fustic; Fustet wood.	Wood of *Rhus Cotinus.*	West Indies; Levant, South Europe.	Fustin or Fisetin.	$C_{15}H_{10}O_6$
Persian Berries; Yellow Berries.	Various species of *Rhamnus.*	Spain, France, Persia, Turkey, etc.	Rhamnetin. Rhamnazin.	$C_{16}H_{12}O_7$ $C_{17}H_{14}O_7$
Annatto.	Pulpy parts of *Bixia Orellana.*	Mexico; South America.	Bixin.	$C_{28}H_{34}O_5$

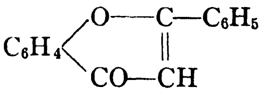
Several of these dyes were obsolete and many others not mentioned here are fully described in "*The Natural Organic Colouring Matters*" Perkin and Everest.

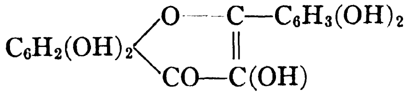
FUSTIC

Fustic is also known under the names Cuba wood and yellow wood, and is the heart-wood of the tree *Morus tinctoria* or *Maclura tinctoria.* It grows in Brazil and tropical America generally, the West Indies, and India; the best qualities being exported from Cuba and Tampico. The tree attains a height of 50–70 feet, and in addition to its use as a dyestuff, the wood is esteemed for cabinet-making purposes.

Colouring Matters.—There are two distinct colouring matters present in fustic,—*morin,* or moric acid, and *maclurin,* or morintannic acid.

Morin, $C_{15}H_{10}O_7$, is the principal colouring matter. It forms pale yellow needles, and is practically insoluble in cold water, but dissolves sparingly in boiling water. It is easily soluble in alkaline solutions. When fused with potassium hydroxide it yields phloroglucinol (*sym.* $C_6H_3OH)_3$), and, like most of the other natural yellow colouring matters, it is a hydroxyl derivative of *flavone,* the constitution of these two compounds being:


Flavone,


Morin.

Morin forms compounds with metals and gives the following reactions.

Alkalies—yellow-brown solution. *Alum*—bright yellow precipitate. *Lead acetate*—orange precipitate. *Copper acetate*—brownish-yellow precipitate. *Ferric chloride*—olive-green coloration or precipitate. *Stannous chloride*—orange precipitate. *Gelatin*—no precipitate.

Maclurin, or Moritannic Acid, $C_{13}H_{10}O_6$.—This substance is a derivative of benzophenone and not of flavone. It is much more soluble in water than morin, and is also readily soluble in alcohol and ether. The ethereal solution fluoresces green and brown. Maclurin melts at 200° and when heated with strong alkali hydroxide solution it yields phloroglucinol, $C_6H_3(OH)_3$, and protocatechuic acid, $C_6H_3(OH)_2COOH$. It dissolves in cold concentrated sulphuric acid with a yellow colour, but is reprecipitated on the addition of water. The solution in concentrated acid deposits brick-red crystals (rufimoric acid) after several days.

A solution of maclurin, on reduction with zinc and sulphuric acid becomes first red and then orange in color. The solution then contains phloroglucinol and *machromin.* The latter substance becomes blue on exposure to air. Ferric chloride produces a violet colour with maclurin which changes to blue. Lead acetate gives a yellow, and stannous chloride an orange precipitate. Gelatin produces a greenish precipitate.

The diazobenzene compound of maclurin is sold commercially under the name of *Fustin*, or Wool Yellow.

Commercial Preparations of Fustic

Fustic, like logwood, is sold as chipped or rasped wood, and as liquid or solid extract, but is now almost exclusively employed in the extract form. Until recently fustic was subjected to the "ageing" process similarly to logwood, but no change corresponding to the conversion of hæmatoxylin into hæmatein takes place, and the only useful action appears to be the incidental one of thoroughly soaking the wood and thus rendering the colouring matter more easily extracted in the dye-bath.

Fustic extracts are manufactured in the same way as logwood extracts. The liquid extracts on standing separate into two layers, the lower layer consisting mainly of insoluble morin, and the upper liquid portion containing most of the maclurin.

Fustic extracts are frequently adulterated with dextrin, molasses, zinc sulphate, alum, tannin extracts, turmeric, or coal-tar dyes, while quercitron extract is of common occurrence. The alum and zinc sulphate are added to enrich the colour of the extract, but they do not really increase the dyeing powder.

Fustic is a mordant-dye and produces with chromium mordants an olive-brown, with aluminium and tin, yellow, and with iron and copper, olive colours. With chromium and aluminium mordants, morin is the only useful colouring matter. With iron mordant maclurin is of chief importance (Gardner, *The Dyer*, 1892, **12**, 46).

Fustic is still largely employed in wool dyeing as the yellow constituent of compound shades. It has the property of easily penetrating thick or felted material. It is little used in cotton dyeing.

Examination of Fustic.—A decoction of fustic has a bitter astringent taste. Alkalies darken the solution to a reddish-brown. Dilute acids make the solution yellower and paler in colour. Sodium aluminate gives a yellow precipitate, stannous chloride or lead acetate produces an orange-yellow precipitate. Ferric chloride gives an olive-brown coloration, and the liquid on standing deposits a dark olive precipitate. The most satisfactory method of examination is that of comparative dye tests carried out on wool mordanted with potassium dichromate. Some samples give much greener shades than others, and such are most esteemed. A more accurate comparison can be arrived at by carrying out the tests on wool previously dyed a pale blue in the indigo vat, since slight differences

in tint are more easily recognised in the case of the green thus produced than with the fustic yellow.

Estimation of Colouring Matter in Fustic Extract.—10 grm. of the dried sample are extracted with absolute alcohol. To the alcoholic solution hot water is gradually added until no further precipitation of morin occurs. The solution is then evaporated to about half its bulk, when most of the maclurin separates out, an addition of hydrochloric acid causing a further precipitation of maclurin. The estimation is only approximate.

Detection of Adulteration in Fustic Extract.—Bruhl (*J. Soc. Dyers and Col.*, 1889, **5**, 124) proceeds as follows:

If present, zinc sulphate and alum may be detected in the solution obtained by extracting the dried extract with nitric acid after gentle charring.

If turmeric is present, unmordanted cotton becomes yellow when boiled in a solution of the sample.

Extract of quercitron may be detected by the much deeper colour while an extract adulterated with this substance produces on wool mordanted with stannous chloride in conjunction with the paler colour which the adulterated extract produces with alum mordant. A sample of fustic extract known to be pure is necessary for comparison.

The wood of the *Osage Orange* tree also contains morin and maclurin and the extract is used in leather dyeing, producing a somewhat purer yellow than old fustic.

WELD

Weld is the dried plant, *Reseda Luteola*, and, although it has now lost much of its importance, it is still cultivated in England, France, Belgium, Italy, etc., to a small extent. The colouring matter, *luteolin*, ($C_{15}N_{10}O_6$) is a flavone derivative and has the composition

$$C_6H_2(OH)_2\left<\begin{array}{l} O\text{---}C\text{--}C_6H_3(OH)_2 \\ \quad\;\;\, \| \\ CO\text{---}CH \end{array}\right.$$

Is thus allied to morin (old fustic), and quercetin (quercitron bark). It forms pale yellow needles and has been prepared synthetically.

Weld produces an extremely bright yellow with alum mordant, and is still used in conjunction with indigo vat blue for producing

certain shades of green. It appears in the market as bundles consisting of the whole of the plant, and before use is generally chopped into small pieces in a chaff cutter.

Quercitron Bark and Flavin

Quercitron bark is the inner bark of a species of oak, *Quercus nigra* or *Quercus tinctoria*, indigenous to the United States. The bark is used in the form of powder or as an extract.

Flavin is obtained by extracting quercitron bark with water at a high temperature, the solution depositing the quercitrin on cooling. This preparation is known as yellow flavin. Red flavin, which is essentially quercetin, is obtained by extracting the bark with dilute acid.

Quercitrin, $C_{21}H_{20}O_{11}$, is the glucoside existing in the bark. Flavin consists essentially of this substance. It forms pale yellow crystals, and on boiling with dilute acid, splits up into the colouring matter, quercetin, and dextrose.

Quercetin, $C_{15}H_{10}O_7$, is one of the commonest yellow colouring matters in vegetable products. It is a simple derivative of flavone having the composition

$$C_6H_2(OH)_2\left\langle\begin{array}{l} O - C - C_6H_3(OH)_2 \\ \qquad\ \ \| \\ CO - C - OH \end{array}\right.$$

and is isomeric with morin (old fustic) and closely allied to luteolin (Weld). The use of quercitron bark and flavin has now almost entirely ceased, but when still employed it is generally in the form of extract.

These dyes are best valued by making comparative dye trials as described under Fustic.

Catechu, Cutch, and Gambier

These are obtained from various species of mimosa, acacia, and areca, growing chiefly in India. They contain large amounts of tannin matter, and varying amounts of catechu-tannic acid (see tannin matters), along with a white crystalline substance, *catechin*. There is also present a brown amorphous oxidation product. These products are used as tannin matters and also for the production of brown shades on cotton.

Catechu-tannic acid constitutes the soluble portion of the product, and has the usual properties of a tannin, giving white precipitates with gelatin and with tartar emetic, and a green precipitate with ferric chloride. On exposure to air, particularly in the presence of alkali, it becomes oxidised to a reddish-brown substance.

The dyeing value of catechu and allied products depends upon the production of the brown oxidation products of catechin and catechu-tannic acid. The only satisfactory method of valuing catechu, cutch, and gambier for use in dyeing is by means of comparative dyeing trials. These should be carried out as follows: 10 grm. cotton yarn is dyed for 1 hour at the boil with 10% of the sample. The material is allowed to cool in the liquid, then taken out, squeezed, and worked in fresh baths for half an hour with 2% potassium dichromate at 80°. A second and third hank should be dyed in a similar way in the same solutions and subsequently chromed, and a parallel series of experiments should be made with the addition of 1% of copper sulphate to the dye-bath. A comparison of the strength and exhausting powers of the samples is thus obtained.

Galloflavin

This yellow dyestuff is obtained by the oxidation of an alkaline solution of gallic acid (see Tannin Matters.) It contains four hydroxyl groups and appears to have the formula $C_{12}H_2O_4(OH)_4$ (Hersig and Tscherne *Monatsh. Chem.* 1914, **35**, 77).

TURMERIC

Turmeric or Indian Saffron is the tuber or underground stem of *Curcuma tinctoria* or *longa* and *C. rotunda*. It is cultivated in India China and southern Asia generally. There is also an African variety. The colour of the roots externally is generally greyish, but in the interior they are usually a deep yellow.[1] When of good quality the roots are hard, give a waxy or resinous fracture and possess a strong aromatic smell and taste.

Pelletier and Vogel (*Annalen*, **44**, 297) state that turmeric contains cellulose, gum, starch, mineral matter, a strong-smelling vola-

[1] The principal commercial varieties of turmeric are: *Chinese*, consisting of many central rhizomes with well-developed branches; *Bengal*, mostly in slender branches of a deep reddish tint; *Java*, which consists of rather small tubers and branches that are often transversely and longitudinally cut; and *Cochin turmeric*, in sections or slices of a larger tuber, some being marked with rather large depressed stem-scars.

tile oil a resinous brown colouring matter and a yellow colouring matter which they named curcumin.

Turmeric powder has a strong odour and a bright orange colour. The taste is bitter and aromatic. Cold water dissolves but little colouring matter, but boiling water extracts a larger quantity. Alcohol dissolves the colouring matter freely, along with the greater part of the resin.

Curcumin, $C_{21}H_{20}O_6$ is prepared according to Jackson and Menke (*J. Amer. Chem. Soc.*, 1882, 4, 77) by treating ground turmeric with petroleum spirit to remove the volatile oil, and then with ether, which dissolves the curcumin together with a large quantity of resin. The product is purified by crystallisation from alcohol. Thus prepared, curcumin crystallises from hot alcohol in thick needles or prisms, which have an orange-red colour and a beautiful blue reflection. Curcumin is odourless when pure, melts at 178°, and is only slightly soluble in water, even when boiling. It is difficultly soluble in cold but more readily in boiling alcohol. The ethereal solution exhibits a strong green fluorescence. It is also soluble in wood spirit and glacial acetic acid, but only slightly so in benzene or carbon disulphide, and is all but insoluble in petroleum spirit. Strong sulphuric acid dissolves curcumin, with a fine reddish-purple colour, gradually changing to black from charring, and the same effect is produced, though more slowly, by strong hydrochloric acid.

Curcumin dissolves readily with a reddish-brown colour in solutions of alkali hydroxides and carbonates, and to a slight extent when boiled with water and calcium carbonate. The ammoniacal solution redeposits curcumin on boiling. On adding a large excess of strong alcoholic potassium hydroxide to a hot alcoholic solution of curcumin, the potassium salt, $C_{21}H_{18}O_6K_2$, separates in globular radiated groups of flame-coloured crystals, which assume a claret colour when dried. This compound is nearly insoluble in ether, but soluble in alcohol and freely so in water. On exposure to air, the alcoholic solution of potassium curcumate assumes a magenta colour, probably from oxidation. When excess of potassium carbonate is added to a hot solution of curcumin in absolute alcohol, the acid salt, $C_{21}H_{19}O_6K$, is formed, and on adding ether this separates in crimson-black flocks resembling magenta.

In consequence of the sensitiveness of curcumin to alkalies, turmeric is sometimes used as an indicator of alkalimetry. The yellow colour is restored by very weak acids, and hence turmeric has been proposed for titrating fatty acids, for which purpose, however, phenolphthalein is better adapted (see vol. II, and R. T. Thomson, (*J. Soc. Chem. Ind.*, 1887, **6**, 195).[1] The alcoholic solution of turmeric exhibits a well marked fluorescence.

The most characteristic reaction of curcumin (and turmeric) is that with boric acid. If an alcoholic solution of turmeric or curcumin be mixed with boric acid, it assumes a deep red colour, distinct from that produced by alkalies. A convenient way of applying the test is to place a small disc of filter-paper, about 1 inch in diameter, in the turmeric tincture, and evaporate the latter to dryness at 100°. On the paper is then poured an aqueous solution of boric acid, or a solution of borax to which sufficient hydrochloric acid has been added to render it distinctly acid to litmus. The red colour is at once developed, or becomes apparent on evaporating the liquid to dryness. On now adding a drop of alkali hydroxide, a very beautiful series of colour of changes will be produced, green and purple being the most prominent. On adding hydrochloric acid a red colour is produced which is again turned green and blue on addition of excess of alkali.

The behaviour of curcumin with boric acid appears to be due to the formation of a substance called by Schlumberger *rosocyanin* (possibly isomeric with curcumin) which may be prepared by treating an alcoholic solution of curcumin with boric and sulphuric acids. The liquid acquires a deep red colour, which changes gradually in the cold, and rapidly on heating, to dark red, orange, and finally to yellow. Hence the operation should be arrested when a sample is found to become blue on adding ammonia. The impure rosocyanin crystallises out as the solution cools. When pure, it forms dark red needles with a green reflection, and is insoluble in water, ether, or benzene. The alcoholic solution has an intense rose-red colour, but rapidly changes. It is turned blue by ammonia, the original colour returning on adding an acid. The alkaline solution becomes grey on exposure to air, and gives blue precipitates with lime or baryta water.

Turmeric is one of the few natural colouring matters for which cotton has a strong attraction. Cotton may be dyed without a

[1] Turmeric is also applicable in the presence of ammonia, to which it is not sensitive.

mordant by heating in a solution of turmeric at 60°. It was formerly much used in wool and silk dyeing both as a substantive dye and as a mordant-dye, and in still so used in India. Turmeric is also employed in paper-staining and for dyeing wood and leather; also as a colouring for butter, cheese, pastry, etc. It is an important ingredient of curry powder.

Powdered turmeric is sometimes adulterated with starch and mineral matters. The *ash* should not exceed 5 to 6%. Common salt is added to turmeric to give it a brighter appearance, but interferes with some of its uses. Turmeric should be quite dry. If damp, it becomes yellowish-brown and is rendered unfit for its chief applications. The characteristics of good turmeric are a rich, deep, but bright, orange colour, and a strong aromatic, rather pungent odour. Turmeric may be valued by dyeings on cotton and on white woollen cloth at 60°, with and without the addition of alum.

On the fibre, turmeric is turned reddish-brown by hydrochloric acid, or an acid solution of stannous chloride, without the solution becoming coloured. Sodium hydroxide and ammonia turn the fibre bright reddish-brown, the solution becoming brownish-orange. Alcohol extracts the colour, producing an orange or yellow solution with green fluorescence. Nitric acid turns the fibre pale yellow.

GAMBOGE

Gamboge is a gum-resin produced by trees growing in various parts of the Malay peninsula. It occurs in cylindrical, hollow, or solid rolls,[1] longitudinally striated on the surface, and either distinct or more or less agglutinated or folded together in masses. Externally it is brownish-yellow, and is covered with a yellow powder. When broken it exhibits a vitreous or conchoidal fracture, the fractured surface being opaque, smooth, glistening, and of a uniform reddish-yellow colour. The powder is bright yellow, and forms a yellow emulsion with water. Although nearly without odour at the ordinary temperature, gamboge evolves a characteristic smell when heated. The taste is at first scarcely perceptible, but after a time it produces a sharp acrid sensation in the throat. Gamboge acts as a drastic purgative.

[1] The cylindrical variety of gamboge is produced by running the juice into bamboo canes. On drying, the gamboge contracts, and consequently holes are often seen through the middle of the cylinders. Inferior gamboge often occurs in irregular masses weighing several pounds.

Gambogin or Gambogic acid, the resin of gamboge, according to Buchner, has the formula $C_{30}H_{35}O_6$. It may be obtained by precipitating the filtered alcoholic solution of gamboge by water, treating the dried precipitate with ether, and evaporating the ethereal solution. The solution is hyacinth- or orange-red, and the powder bright yellow. It softens on heating and melts at 75°–80°, solidifying to a glassy mass on cooling. It is tasteless, and, according to Hurst, has no purgative action. Gambogin is readily soluble in alcohol, ether, and chloroform, but is only slightly soluble in petroleum spirit. It has well-marked acid properties, decomposing carbonates of the alkali metals at a boiling heat. It dissolves in alkali hydroxides with an orange-red colour, and is precipitated in gelatinous flakes on acidifying the solution. On adding excess of common salt to the solution of gambogin in sodium hydroxide, the sodium salt is thrown down as a red precipitate.

The gum of gamboge, is a transparent, brownish mass, having a sweetish taste and slightly adhesive properties. It is soluble in water forming an opalescent solution, which is rendered clear by acids, and is not precipitated by basic lead acetate, ferric chloride, mercuric chloride, borax, or alcohol. It appears to be a glucoside.

Gamboge dissolves in alcohol, in ether, and in ammonia. The ammoniacal solution produces a red precipitate with salts of barium, yellow with those of zinc, reddish-yellow with lead acetate, and brownish-yellow with silver nitrate.

The following analyses by Christison indicate the composition of commercial gamboge:

	Pipe gamboge from Siam		Cake gamboge from Siam		Ceylon gamboge			
Resin	74.2	71.6	64.3	65.0	68.8	71.5	72.9	75.5
Gum	21.8	24.0	20.7	19.7	20.7	18.8	19.4	18.4
Starchy matter	......	......	6.2	5.0	......	......	......	......
Woody fibre	......	......	4.4	6.2	6.8	5.7	4.3	0.6
Moisture	4.8	4.8	4.0	4.6	4.6	......	......	4.8
	100.8	100.4	99.6	100.5	100.9	96.0	96.6	99.3

A sample of gamboge analysed by Hurst (*Pharm. J.*, 1889, [3], **19**, 761) contained: moisture, 2.50; mineral matter, 1.05; resin, soluble in ether, 66.05; wax, soluble in alcohol, 4.31; and gum, 26.03%; total, 99.94%.

Commercial gamboge is liable to adulteration with *mineral matters* and *starch*. The *ash* should not much exceed 1%. Starch may be detected by exhausting with alcohol, boiling the residue with water, and adding iodine to the cooled liquid, when the well-known blue colouration will be produced if starch be present. It will be observed that the analyses of Christison of cake gamboge from Siam show a small proportion of starch.

Gamboge is not employed as a dye. It has a limited use in medicine as a purgative, and is employed as a yellow pigment in water-colour painting.

SAFFRON

Saffron consists of the stigmata of the flowers of *Crocus sativus*, of which about 500,000 are required to produce 1 pound weight. It has an agreeable odour, and a bitter pungent taste.

An essential oil is obtained by distilling saffron with water in a current of carbon dioxide, agitating the distillate with ether, and evaporating the ether in an atmosphere of carbon dioxide (Kayser, *J. Soc. Dyers and Col.*, 1885, **1**, 43). It is a very mobile, nearly colourless liquid of the terpene ($C_{10}H_{16}$) class, having an intense odour of saffron, and very prone to absorb oxygen and become thick and brown.

If saffron is treated with ether, to remove the fat and essential oil, and the residue treated with cold water, the colouring principle, *crocin*, is dissolved. On shaking this solution with purified animal charcoal the colour is rapidly absorbed, and on filtering and boiling the charcoal with rectified spirit, it again passes into solution. The filtered liquid yields crocin on evaporation.

Crocin is a glucoside and has the formula $C_{44}H_{70}O_{28}$ (?). It forms a yellowish-brown mass, the powder of which is yellow. It dissolves readily in water and dilute alcohol, but with difficulty in absolute alcohol or ether. Concentrated sulphuric acid dissolves it with a blue colour, changing to violet, then to cherry-red and finally to brown. Concentrated nitric acid also gives a blue colouration, changing to brown.

When crocin is hydrolysed it forms the colouring matter *crocetin*, $C_{34}H_{46}O_9$. The latter is best prepared by heating crocin with dilute hydrochloric acid in a current of carbon dioxide, when crocetin is precipitated as a red powder, scarcely soluble in water, but soluble in

presence of an alkali with an orange colour, and reprecipitated on adding an acid.

A second glucoside, *picrocrocin*, $C_{28}H_{66}O_{17}$, is said to be obtained in prismatic crystals, melting at 75°, and soluble in water and alcohol, on extracting dried saffron with ether for a prolonged period. When hydrolysed, it splits up into a sugar and a terpene oil of a saffron-like odour.

Saffron is employed for colouring pastry, and has a limited use in medicine. It is liable to various substitutions and adulterations, which are classified by J. M. Maisch (*Analyst*, **10**, 200) as those derived from the same plant and those coming from other sources. The stigmata of which genuine saffron consists become thinner toward the leaves, terminate in a yellow thread, and three are generally united. Saffron *styles* are present in all saffron of Spanish origin to a greater or less extent, and crocus *stamens*, dyed so as to resemble the stigmata, are also met with. The *corolla tubes* of the crocus, dyed with Brazilwood or santal-wood, are said to be frequently used for adulterating saffron. Various other coloured vegetable products are referred to by Maisch, including dyed *calendula* florets (marigold), and this may be detected by treating the suspected portions of the sample with petroleum spirit, which is not coloured by genuine saffron, but produces a citron-yellow colour with the adulterant.

Safflower and *red poppy* have also been observed as adulterants of saffron. In the latter case the infusion is turned greyish-green by ammonia and bright red by nitric acid. Safflower is said to have been so commonly substituted for saffron in some parts of America that the genuine substance was unknown (*Pharm. J.*, 1876, [3], **5**, 950).

Mineral additions, such as chalk, gypsum, barium and sodium sulphate, etc., have been observed as adulterants of saffron, being made to adhere by means of honey, glucose, or glycerin. The ash of genuine saffron of good quality ranges from 4 to 7%, but in samples of Alicante saffron, Hanbury (*Pharm. J.*, 1870 [3], **1**, 241) found ash varying from 12 to 28%, the excess being due to mineral adulterants. Ingham has described a sample of saffron containing 45% of mineral impurity, besides a quantity of stamens of the common crocus; Hart, a saffron yielding 20% of ash, the greater part of which consisted of barium sulphate; and Tanner, a sample containing a considerable quantity of a red ferruginous earth. Adrian has

described a saffron yielding 26.4% of ash, containing borate, chloride, sulphate of sodium, and potassium carbonate, the last having probably been derived from tartrates. The presence of ammonium nitrate was also suspected.

Grispo found vegetable filaments of unknown origin in saffron, together with water, glucose, and barium sulphate. Kanoldt examined a fictitious saffron that consisted entirely of an alga, probably *Fucus amylaceus*, which had been weighted with a coloured mixture of chalk and honey. For the testing of saffron see Dowzard, *Pharm., J.*, 1898, **4**, 443; Viuassa, *Arch. Pharm.*, 1892, **231**, 353 and Nestler, *Zeit. Nahr. Deut.*, 1892, **6**, 489.

If genuine saffron be scattered on the surface of warm water, it immediately expands into a characteristic form, readily distinguishable from crocus stamens, or the florets of safflower, marigold, or arnica.

Saffron gives a fine yellow colour on silk, but is now rarely if ever used as a dye. It is still employed in medicine and in cooking.

ANNATTO

Annatto, occasionally called *arnotta* and *rocou*, is composed of the pulp surrounding the fruit of *Bixa orellana*, growing in the East and West Indies and South America. The two chief kinds are Spanish annatto, imported from Brazil, and the flag or French annatto which comes from Cayenne. Brazil annatto occurs in cakes or rolls, is hard and dry, brownish on the exterior but red inside, and has a rather agreeable odour. Cayenne annatto is a soft paste, of a bright yellow colour. It should contain 10 to 12% of the pure dye and not more than 5 per cent. of ash. It often has a repulsive urine-like odour, said to be due to the actual addition of urine to keep it moist and impart a rich colour.

Annatto contains two yellow colouring matters, *bixin* and *orellin.*

Bixin, $C_{28}H_{34}O_5$, (Etti) the properties and chemical relationships of which have been examined by Heiduschka and Riffazt (*Arch. Pharm.*, 1911, **240**, 43), van Hasselt (*Rec. trav. chim.*, 1914, **33**, 192), Herzig (*Monatsh*, 1914, 997) and others may be prepared by digesting annatto at about 80° with alcohol and sodium carbonate. The filtered liquid is treated with half its volume of water and a saturated solution of sodium carbonate. The precipitate, consisting of the sodium salt of bixin, is purified by solution in weak alcohol

and precipitation by sodium carbonate, and is then decomposed by hydrochloric acid. Bixin forms minute yellow leaflets which melt at 176°. It is insoluble in water and only slightly soluble in alcohol, benzene, carbon disulphide, or acetic acid, but is readily soluble in ether. Bixin forms a sodium salt, $C_{28}H_{33}O_5Na$, $2H_2O$, which crystallises in lustrous red needles, soluble in water, but insoluble in alcohol and ether. It also yields a compound, $C_{28}H_{32}Na_2O_5$, $2H_2O$, which forms a dull red powder. Bixin dissolves in strong sulphuric acid with a bright blue colour, and on dilution with water a dark green precipitate is formed.

Orellin is a yellow substance soluble in water and alcohol, but insoluble in ether, and dyes cloth mordanted with alum, yellow. It is probably an oxidation-product of bixin.

Annatto is only partially soluble in water, but more completely so in alcohol. It dissolves readily but sometimes imperfectly in solutions of alkali hydroxides and carbonates, of borax, and of soap, forming liquids of orange or red colour, which furnish orange-red precipitates with acids. It gives orange lakes with alumina and ferrous sulphate, a yellowish-brown precipitate with salts of copper, and a lemon-yellow with tin salts. Concentrated sulphuric acid dissolves annatto with a deep blue colour, which gradually changes to green and violet. On adding water a deep green precipitate is formed.

Samples of annatto have been found adulterated with ochre, brick dust, sand, chalk, salt, starch, gum, turmeric and other colouring matters. It is chiefly used in the colouring of butter and cheese.

RED DYESTUFFS

Cochineal, Kermes and Lac Dye.—Apart from the now extinct Tyrian Purple these are the only colouring matters of importance which are of animal origin. Cochineal is the female of the *coccus cacti*, an insect which feeds on various species of cactus, and is collected largely in Mexico, Guatemala, the Canary Islands, and Java.

The insects, which have no wings, are merely brushed off the plants and killed by stoving or steaming. The insect is dark reddish-brown in colour, and in appearance and size resembles the common ladybird. The natural appearance of the insect may usually be observed by allowing a few cochineal grains to soak for some time in water.

"Silver-grey" cochineal is produced by stove killing, "black" cochineal by steam killing, the latter removing the grey powder to which the appearance of the former is due.

The dyestuff undergoes absolutely no preparation for the market, but before being employed the insects are ground to powder.

Cochineal is relatively rich in colouring matter compared with most of the other natural dyes, containing from 10 to 20% of the pure substance. The latter exists in the dried insect (principally in the eggs), as a glucoside, *carminic acid*, from which the real colouring matter, *carmine red*, is readily produced.

Carminic acid, $C_{22}H_{20}O_{13}$ (Dimoth *Annalen*, 1913, **399**, 1) is an anthraquinone derivative. It crystallises in red prisms, is soluble in water, alcohol, or benzene, but insoluble in ether. It gives no definite melting point being decomposed on heating. It is readily hydrolysed by boiling with dilute acid, producing carmine red.

Carminic acid is found in several other insects and also in some plants, *e. g.* the *monada* and *dydima.*

Carmine red, $C_{11}H_{12}O_7$, is obtained by boiling the diluted aqueous solution of carminic acid with a few drops of mineral acid. It forms a dark purplish amorphous substance which produces colour lakes of very varied hues with different metals. The most characteristic are those with the following metals: *Tin*, bright scarlet; *aluminium*, crimson; *chromium*, purple; *iron*, bluish-purple; *copper*, brown; *uranium*, grey.

When treated with nitric acid carmine red produces *nitro-coccusic acid*, $C_8H_5(NO_2)_3NO_3$, along with oxalic acid.

A saponifiable fat, *coccerin*, varying in amount from 1 to 4%, also exists in cochineal.

Although for many purposes cochineal has been replaced by artificial red dyes, it is still used to a considerable extent in the production of scarlet cloth. It is also largely employed in the preparation of cochineal carmine, an artists' pigment which is very stable under the action of light.

Ammoniacal cochineal consists of *carminamide*, $C_9H_9O_4N$, (Schutzenberger) an amino-compound of carmine red, and is produced by allowing ground cochineal to remain in contact with ammonia for several days. It dyes a beautiful purple colour in conjunction with tin mordant, but is now obsolete.

Examination of Cochineal.—Genuine samples of cochineal vary considerably in colouring power. Cochineal is not now adulterated to the same extent as was the case when it was the chief scarlet dye available. A silver-grey appearance and additional weight was sometimes given to black cochineal by covering it with barium sulphate.

Another mode of adulteration is to extract part of the colouring matter by boiling the insects in water and then re-drying.

The relative values of samples of cochineal are best estimated by a comparative dye trial, using wool previously mordanted with 4% stannous chloride and 4% cream of tartar; about 5% of the dyestuff being the most suitable amount. A satisfactory colorimetric method may, however, be carried out as follows: 0.25 grm. of each sample is finely powdered and boiled with 200 c.c. of alcohol for 15 minutes, then cooled and made up to 250 c.c. with alcohol. 5 c.c. of the filtered solution along with 1 c.c. of a 1% solution of alum is diluted to 100 c.c. with water and the relative intensity of colour is estimated. The full colour develops in 2 to 3 minutes.

Mineral matter should be estimated, genuine samples containing less than 1%.

A decoction of cochineal is used to impart a pink colour to confectionery and other foodstuffs. To detect cochineal in alimentary substances, the substance should be dissolved in water or weak alcohol rendered faintly acid with acetic acid. The liquid is then agitated with amyl alcohol, separated and evaporated in presence of water. The aqueous solution obtained is treated with a few drops of a 3 per cent. solution of uranium acetate, when a beautiful bluish-green colouration or precipitate will be produced if cochineal be present. Acids destroy this colour, with production of the orange tint of the carminic acid. In the case of wine, the amyl alcohol employed should be mixed with an equal volume of benzene, or, preferably, toluene, as otherwise œnolin will also be taken up, and will mask the reaction of the cochineal. Ammoniacal cochineal, which has been occasionally employed to colour wine, produces a rose-violet or violet-blue lake with uranium oxide. Logwood gives a somewhat similar reaction, but may be distinguished from cochineal by the production of a purple tin-logwood lake; cochineal producing a bright scarlet tin-lake.

Cochineal carmine or carmine lake is a brilliant red pigment produced by precipitating a decoction of cochineal with alum or stannic chloride with addition of acid oxalate or tartrate of potassium. The employment of a decoction of cochineal itself, and not of carminic acid, is also a necessary condition, the nitrogenous matters being essential to its formation.[1] A sample examined by C. Liebermann (*J. Soc. Dyers and Col.*, 1885, **1**, 269) contained, after drying, 3.7% of nitrogen, only 0.25% of which could be expelled by boiling with dilute alkali. The remainder appeared to exist as proteins, or probably in part as tyrosine.[2] The ash was white, and amounted to 8.1%. 100 parts contained 43 of alumina and 45 of lime, 0.67 of tin oxide, and small proportions of magnesia, alkalies, and phosphoric acid. The composition of the original carmine was probably approximately: water, 17; mineral matter, 7; nitrogenous matters, 20; and colouring matter, 56%; with traces of wax.

Cochineal-carmine is liable to adulteration with starch, kaolin, vermilion, red-lead, chrome-red, etc. These admixtures may be detected by treating the sample with dilute ammonia, in which a pure sample should be completely and readily soluble. The solution of cochineal-carmine in ammonia yields no precipitate with ammonium oxalate, and the precipitate produced on adding an acid is a lake from which the colouring matter can only be set free by heating with moderately concentrated mineral acid. If the ammoniacal solution of carmine be heated on a water-bath, with constant stirring, until entirely destitute of ammoniacal odour, the product is a deep ruby-red liquid which gives no precipitate with mercuric chloride, and becomes purplish on addition of ammonia. *Vermilionette*, an eosin lake, can be recognized by treating the colouring matter with dilute sulphuric acid and agitating the liquid with ether, which on evaporation will leave the eosin in a condition ready for further examination.

Commercial cochineal-carmine contains: colouring matter 30 to 65 aluminium and lime 5 to 12, and moisture 2 to 20%.

Carmine is employed by artists, paper-stainers, and textile-printers.

[1] Several recipes, collected from standard works, have been published by M. Dechan (*Pharm. J.*, [3], **16**, 611). Tne English process is said to consist in boiling 1 lb. of cochineal and ½ oz. of potassium carbonate with 7 gallons of water for 15 minutes. The heat having been withdrawn, 1 oz. of powdered alum is added, and the liquid stirred and allowed to settle. The clear liquid is decanted, ½ oz. of isinglass added, and heat applied till a coagulum forms, when the liquid is stirred briskly and allowed to settle.

[2] As albumin and gelatin are sometimes employed in preparing carmine, it does not follow that the whole of the nitrogen present had its origin in the cochineal.

Kermes is one of the most ancient dyes known; its use long antidating that of cochineal. Under its Hebrew name "tola" and its Greek and Latin designation "coccus" it is frequently mentioned in Scripture and other ancient writings.

Kermes is an insect found on the kermes-oak (*Quercus coccifera*). It possesses about one-tenth the colouring power of cochineal. Kermesic acid ($C_{18}H_{12}O_9$), the colouring matter, forms brick-red crystals which differ from carminic acid in being soluble in ether. It appears to be an anthraquinone derivative. It produces very similar shades to cochineal, beautiful in tone and of excellent fastness, and imparts to wool dyed with it a faint aromatic scent. It is now quite obsolete.

Lac-dye is the product of *Coccus lacca*, which lives on the banyan and other trees, on the twigs of which the ova are deposited. From the mature and impregnated female insects a resinous substance exudes, which encloses the eggs. The twigs, with the attached resin are sold as *stick-lac*. If the resinous concretion be removed, powdered, and triturated with water, the greater part of the colouring matter dissolves, and the residue when dried is known as *seed-lac*. If this be melted and squeezed through cotton, it yields *shell-lac* or *shellac*, (see vol. 4, p. 67). The following figures by Hatchett indicate the relative composition of these three lacs:

	Stick-lac	Seed-lac	Shell-lac
Resin	68.0	88.5	90.9
Colouring matter	10.0	2.5	0.5
Wax	6.0	4.5	4.0
Gluten	5.5	2.0	2.8
Foreign bodies	6.5	.	..
Loss	4.0	2.5	1.8
	100.0	100.0	100.0

Lac-dye is prepared by treating stick-lac with a weak alkaline solution and precipitating with alum, or with lime to which some alumina has been added.

The colouring matter of lac-dye has been investigated by R. E. Schmidt (*J. Soc. Dyers, and Col.* 1887, **3**, 122), who terms it *laccaic acid*, and points out its non-identity with, but close resemblance to, carminic acid.

Laccaic acid, $C_{20}H_{14}O_{10}$ (Dimroth, *Annalen* 1913, **399**, 62) forms a brownish-red crystalline powder or crust, appearing under the microscope is well formed rhombic tables. It melts without decom-

position at 180°. It is abundantly, though slowly, soluble in alcohol, and freely soluble in methanol, amyl alcohol, and glacial acetic acid. It is somewhat less soluble in water, with a bluish-red colour, and is insoluble in benzene and petroleum spirit. It resembles carminic acid in being nearly insoluble in ether, but is not precipitated on adding ether to its alcoholic solution. It is a well-defined dibasic acid, and is its reactions and the absorption-spectrum of its alkali-metal salts closely resembles carminic acid; but a difference exists between the absorption-spectra of the two substances when dissolved in conc. sulphuric acid.

Schmidt gives the following results obtained by the analysis of 2 samples of lac-dye:

	1	2
Moisture (expelled at 100°)	9 0	11.26
Mineral matter	15.7	18.24
Colouring matter	10.4	13.20
Other organic matter	64.9	57.30
	100.0	100.00

A good lac-dye should be soft enough to be broken with the fingers, and should powder readily under the pestle. The fracture should be deep in colour, not shining and resinous. When breathed on, it should emit a strong and characteristic odour. Samples which are hard and have a resinous fracture are usually poor in colouring matter, and contain an excessive proportion of resin. The amount of this constituent may be judged from the bulk of the precipitate produced on diluting the alcoholic solution of the lac with water.

A superior variety of lac-dye is obtainable by treating stick-lac with weak ammonia and adding stannous chloride to the solution, when the colouring matter is thrown down as a fine red tin-lake. A lake is also obtained by substituting sodium hydroxide and alum for the ammonia and tin salt in the above process. Lac-lake usually contains about 50% of colouring matter, 40 of resin, 9 of alumina, and 1% of impurities.

Lac-dye gives much the same colour as cochineal, but two or three times the quantity is requisite to produce the same intensity of shade.

ORCHIL AND CUDBEAR

Purple, brown and yellow dyes derived from lichens have been in use from time immemorial. Various species of lichens have also

been used in medicine and as foods. A considerable number of species of lichen are used in the manufacture of orchil and cudbear, the chief being *Roccella tinctoria*, known as Valparaiso weed, and *R. fuciformis*, or Lima weed. Other species are collected in Sweden and in the Auvergne district.

The lichens do not contain any ready formed colouring matter but certain colourless compounds, from which colouring matter is produced by the action of ammonia and air. The principal colour-producing compounds existing in the lichens are *erythrin*, $C_{20}H_{22}O_{10}$, *lecanoric acid*, $C_{16}H_{14}O_7$, and *evernic acid*, $C_{17}H_{16}O_7$. These all yield the colouring principle *orcinol*, $C_7H_8O_2$, from which the colouring matter, *orcein*, $C_{28}H_{24}N_2O_7$, is directly produced. A full account of the chemistry of the various lichen products will be found in *The Natural Organic Colouring Matters*. Perkin and Everest p. 529 *et. seq.*

Orcinol. Orcin. 3 : 5-dihydroxy-methylbenzene, $C_6H_3(CH_3)(OH)_2$.—This substance is homologous with resorcinol. It forms six-sided monoclinic prisms, melting at 58° and containing 1 mol. H_2O. The crystals effloresce gradually over sulphuric acid, and more rapidly when heated to 100°. The anhydrous substance melts at about 107°, and distils with some decomposition at about 287° under atmospheric pressure, but may be obtained pure and colourless by distillation *in vacuo*. When pure, orcinol is colourless, but it acquires a pale reddish-brown colour on exposure to air. It has an intensely sweet, but unpleasantly astringent taste. Orcinol is extremely soluble in hot water, but much less so in cold. It is almost completely precipitated in fine needles when its concentrated solution is warmed with saturated brine. Orcinol dissolves readily in alcohol and ether, but less easily in hot benzene. The crystals deposited from the ethereal solution are anhydrous. It is neutral in reaction, but possesses marked acid properties. It readily decomposes sodium carbonate, and precipitates silica from silicates.

With oxidising agents orcinol yields oxalic acid. With concentrated sulphuric acid it gives a sulphonic acid. When treated with a solution of bleaching powder, it yields an intense purple-red coloration, which rapidly changes to yellow. The most minute trace of orcinol may be detected by this test.

If an alkaline solution of orcinol be heated with a little chloroform, it becomes first purple-red and then bright red, and on dilution with

water exhibits an intense greenish-yellow fluorescence, from the formation of homofluorescein, $C_{23}H_{18}O_5$. This reaction (Schwartz *Ber.*, 1880, **13**, 543) is so delicate that the compounds which yield orcinol on treatment with alkalies can readily be detected by this means in the lichens containing them, by simply boiling a few fragments of the plant with a 5% solution of potassium hydroxide, adding a little chloroform to the clear liquid, then warming the solution for ten minutes and diluting it with water.

An aqueous solution of orcinol is not precipitated by mercuric chloride, lead acetate, cupric sulphate, tannin, or gelatin. With basic lead acetate it yields a white precipitate, and with ferric chloride a violet-black coloration or red precipitate.

On addition of bromine-water to orcinol in aqueous solution, tribromorcinol, $C_7H_5Br_3O_2$, is formed, and the reaction has been recommended by Heymann for the quantitative estimation of orcinol and the assay of the lichens. The process is carried out exactly as in the volumetric estimation of phenol by bromination.

Orcein, $C_{14}H_{12}N_2O_3$, is the product of the action of ammonia and oxygen on orcinol. It forms a brown amorphous mass, having a beetle-green lustre. Orcein is somewhat soluble in water with a red colour, but is reprecipitated from its solution by neutral salts of the alkali-metals. In ether it is insoluble, but dissolves readily in alcohol, yielding a scarlet solution. In alkalies orecin dissolves with formation of splendid purplish-violet solutions.

Manufacture of Orchil.—The weeds are torn into small fragments and placed in iron boilers with a dilute solution of ammonia. The temperature is kept at 35°–45° during from 5 to 7 days. The fermentation which ensues results in the production of orcinol, which is finally converted into orcein. The process is controlled by withdrawing samples and testing from time to time. If the fermentation proceeds too far, the colouring matter is destroyed.

The product still containing the weed residue is known as orchil paste. Orchil liquor is obtained by straining off the worthless solid residue. It is liable to ferment on storage.

Cudbear is produced by evaporating orchil paste to dryness and grinding.

Orchil and cudbear are still used to a considerable extent on wool and silk, and may be applied either without or with a mordant, and in a neutral or acid bath.

Examination of Orchil and Cudbear

Different samples of orchil or cudbear may vary much in strength, brilliancy, and hue, without being purposely adulterated. Comparative dyeing trials carried out on woollen yarn or cloth are the most satisfactory method of valuation. Dye tests should be made both in neutral and acid solution, using 3 to 5% of the dye. In the case of the neutral dyeings, about 3% of sulphuric acid should be added after removing the wool, and a second piece of wool dyed in the same vat, this rendering evident any adulteration with an acid coal-tar dye. The degree of exhaustion of the baths frequently varies much with different samples and should be investigated either by successive dyeings or by colorimetric examination of the waste dye liquors.

The most frequent adulteration is the coal-tar dye, magenta, which may be detected as follows (Breinl, *J. Soc. Dyers and Col.*, 1888, **4**, 46, improved by Rawson, *ibid.*, 1888, **4**, 68):

2 grm. of cudbear, or 4 grm. orchil liquor are dried and boiled with 50 c.c. alcohol for 15 minutes, and then diluted with 100 c.c. of water. 20 c.c. of a solution of basic lead acetate (sp. gr. 1.25) followed by 20 c.c. strong ammonia are added. After shaking, the solution is filtered, and the precipitate washed with a solution containing 1 part ammonia, 5 parts alcohol, and 10 parts water.

With pure cudbear the filtrate remains colourless on acidifying with acetic acid, whereas if magenta is present, a strong red colour is immediately developed. The amount is estimated by comparison with a standard solution of magenta.

This process is also applicable for the detection of Methyl Violet and Safranine, which may be distinguished by dyeing a small thread of wool in the solution and applying tests for those colouring matters.

Breinl, has also studied the reactions of a number of coal-tar colours similar in shade to orchil. Kertesz (*J. Soc. Dyers and Col.*, 1885, **1**, 217) tests for Acid Magenta as follows:

A small quantity of the sample is boiled with water and filtered. The filtrate is mixed with a little benzaldehyde, and stannous chloride and hydrochloric acid are added. On shaking and allowing the mixture to stand, the lower layer of liquid will appear coloured if Acid Magenta is present.

Liebmann and Studer detect Magenta and Acid Magenta as follows:

1 grm. of the dye is boiled with 100 c.c. of water and after cooling saturated with SO_2. Acetone is then added, when, if magenta or Acid Magenta is present, a violet colour gradually develops.

LITMUS

Litmus.—This product is allied to orchil. It is prepared from various species of *Rocella*, *Variolaria*, and *Lecanora* by allowing them to ferment in presence of ammonia, as in the manufacture of orchil, except that, in the case of litmus, potassium carbonate is likewise added. When the mass has become violet, stale urine, lime, and potassium hydroxide are added, and the mass is again allowed to ferment until it assumes a blue colour, when it is mixed with chalk or gypsum and a little indigo, and made up into small tablets.

Commercial litmus contains several colouring matters. On extracting with cold alcohol, a red solution is obtained, which is unaffected by acids. On treatment with water, filtering, evaporating the solution to dryness and treating the residue with absolute alcohol and a little acetic acid, a scarlet colouring matter is removed, which is changed to purple by ammonia, while the pure litmus-blue remains behind as a brown powder, soluble in water to a reddish-brown solution, which is turned blue by the slightest trace of an alkali.

Azolitmin, $C_7H_7O_4N$, the characteristic colouring matter of litmus, may be obtained in a state of purity, according to de Luynes, by digesting 1 part of orcinol with 1 of strong ammonia, 25 of crystallised sodium carbonate, and 5 of water, at 60°–80° for 4 or 5 days in a closed vessel. A blue liquid is thus obtained, which is diluted with water and slightly acidified with hydrochloric acid, when a precipitate is formed, which, after being washed and dried, is regarded as pure azolitmin.[1] So obtained, azolitmin is a reddish-brown powder, which is only slightly soluble in water and insoluble in alcohol and ether. It appears to have the characters of a weak acid, the salts of which are blue, and the potassium compound of which exists in litmus.

[1] Kane prepares azolitmin by exhausting powdered litmus with water, mixing with clean fine sand and evaporating on a water-bath. Sufficient hydrochloric acid is added to give a red solution after the carbon dioxide has been driven off, and the evaporation is continued to dryness. The residue is washed with water and again evaporated on a water-bath, after which the sand is freed from its coating of pure azolitmin by treatment with weak ammonia, and the azolitmin is finally obtained from its solution by precipitation with sulphuric acid.

Litmus exhibits a very characteristic absorption-spectrum. Ether extracts it from an acid solution, and forms a yellow liquid, which shows absorption in the more refrangible end of the spectrum to a point midway between *D* and *E*. On adding a drop of ammonia to the ethereal solution the liquid becomes blue, and an absorption-band is formed, which commences at *d*, where it is extremely dense, and gradually diminishes to *E*. On shaking the ethereal solution with ammoniacal water, the colouring matter passes into the aqueous liquid and the blue solution shows a well-marked absorption-band at *D*. Addition of acid now changes the colour to red, and the band at *D* disappears, the spectrum of the acidified liquid resembling that of œnolin, the colouring matter of red wine.

Litmus is not employed in dyeing or calico-printing, but is used for colouring wine and vinegar, and in the laboratory is well known as an indicator of neutrality (v. Acidmetry and Alkalimetry).

Litmus gives a deep blue colour with alkalies and a red with acids; alkaline carbonates also produce a blue colour. As litmus is sensitive to carbonic acid and hydrogen sulphide, when carbonates or sulphides are titrated in its presence, the carbonic acid and hydrogen sulphide gases which are liberated must be driven off by boiling before the end-point is taken. Litmus is a good indicator for titrating the acids present in the normal salts of such alkaloids as quinine, strychnine, morphine, narceine, and papaverine, since these are neutral to litmus. The alkaloids caffeine, narcotine, and theobromine are also neutral to litmus, but their salts act like a corresponding amount of the free acid. Aniline, toluidine, and quinoline exhibit a neutral reaction toward litmus, and hence cannot be titrated by its aid.

Tournesol en drapeaux is a blue colouring matter allied to litmus manufactured from the *Croton tinctorium*. It is used for colouring the wrappers of cheeses and the change of colour from blue to red serves to indicate the point when the cheese becomes "ripe" by the development of lactic acid.

MADDER

Before the introduction of artificial alizarin in 1868, madder was the most important of the natural dyestuffs, with the exception of indigo. It has now been almost entirely replaced by artificial alizarin, and is used only to a very limited extent.

Madder is the ground root of the *Rubia tinctoria*, or other allied plants. The roots are dug up, ground, and stored for some time to develop the colouring matter.

The colour-producing substance exists in the root in the form of a glucoside, $C_{26}H_{28}O_{14}$, named by Schmuch *rubian*, but later called *ruberythric acid*. This substance is sparingly soluble in cold, and more easily in hot water, alcohol, and ether. During storage, or in the dye-bath, it undergoes hydrolysis, this being brought about by the action of a specific enzyme *erythrozyn*.

The chief colouring matter is alizarin, $C_{14}H_8O_4$, but small quantities of purpurin, $C_{14}H_8O_5$, are also formed. Both these colouring matter (as also a large series of derivatives from them) are now produced in large quantities by synthetic processes. (See Coal-tar Colours.)

Samples of madder are now so rarely met with that it is unnecessary to enter into detail with regard to their analysis. The comparative value is best estimated by dye trials carried out on mordanted wool or cotton. In the case of wool, tests should be made both on potassium dichromate and on alum mordants.

Garaneine was produced from madder by boiling with dilute sulphuric acid to hydrolyse the indican. It was mainly used in calico printing. *Extract of Madder* (Penrod) was obtained by extracting garaneine with water and allowing the colouring matter to deposit from the solution.

REDWOODS

The so-called red dye woods are now almost obsolete. They are divisible into 2 groups—the *soluble* and *insoluble* redwoods. The former group comprises Brazil or Permanbuco wood, peach wood, Lima wood and sapan wood. These appear all to contain the same colouring principle *brazilin* $C_{16}H_{14}O_5$ which by "ageing" (oxidation) is converted into the colouring matter *brazilein* $C_{16}H_{12}O_5$, the change being analogous to the conversion of hæmatoxylin into hæmatein. (See logwood p 388.)

The soluble redwoods are mordant dyes and produce purple shades with chrome mordant, and crimson with alum. These woods are still imported to a considerable extent, but are chiefly used in making pigments and not in dyeing.

REDWOODS

Commercial name	Botanical origin	Geographical origin	Colouring principle	
			Name	Formula
Soluble Redwoods.	...	...	...	...
Brazil wood; Pernambuco wood.	Wood of *Cæsalpinia Brasileinsis* and *C. Crista.*	Brazil, Perambuco, Jamaica.	Brazilin. Brazilein.	$C_{16}H_{14}O_5$ $C_{16}H_{12}O_5$
Peach wood.	Wood of *Cæsalpinia echinata.*	Nicaragua, Sierra Nevada.	Brazilin. Brazilein.	$C_{16}H_{14}O_5$ $C_{16}H_{12}O_5$
Sapan wood.	Wood of *Cæsalpinia sapan.*	Siam, Japan, East Indies, etc.	Brazilin. Brazilein.	$C_{16}H_{14}O_5$ $C_{16}H_{12}O_5$
Insoluble Redwoods.	...	...	...	...
Sandal, Santal, or Sanders wood.	Wood of *Pterocarpus santalinus.*	East Indies, Ceylon, Madagascar.	Santalin. Deoxy-santalin.	$C_{24}H_{22}O_8$(?) $C_{24}H_{24}O_7$(?)
Barwood.	Wood of *Baphia nitida.*	Sierra Leone.	Santalin.	...
Camwood, or Kambe wood.	Wood of species of *Pterocarpus.*	West coast of Africa.	Isosantalin.	...

The insoluble redwoods comprise camwood, barwood, and sanderswood. The only application of these woods in dyeing is that of "bottoming" indigo blues, the wool being boiled for some time with the wood before being dyed in the indigo vat.

The colouring matters of these woods do not appear to be identical, but they have not been thoroughly investigated. Samples may be examined by dye trials made on wool mordanted with 2% of potassium dichromate.

ALKANET

This consists of the cortical parts of the root of *Anchusa tinctoria.* The colouring matter *alkannin* is best obtained pure by extracting alkanet root with dilute potassium hydroxide solution, and agitating the solution with ether to remove a reddish-brown impurity. On saturating the alkaline liquid with carbon dioxide the alkannin is precipitated, and may be purified by solution in ether.

Alkannin, Anchusin, or *Anchusic Acid.*—This compound (probably either $C_{15}H_{14}O_4$ or $C_{15}H_{12}O_4$) is a reddish-brown resinous substance of metallic lustre. It is insoluble in water, but soluble in alcohol, glacial acetic acid, ether, chloroform, carbon disulphide, turpentine, and fixed oils. The alcoholic solution is crimson, and is unchanged by exposure to light or by continued boiling. It gives a blue colora-

tion with alkalies (restored to crimson by acids),[1] a bluish-violet precipitate with aluminium acetate, a crimson precipitate with stannous chloride, and a purple precipitate with stannic chloride. Lead acetate produces a blue, and iron salts a violet precipitate. Alkannin forms a diacetyl derivative which crystallises from glacial acetic acid in brownish-yellow grains. It is evidently a derivative of methyl-anthracene, $C_{15}H_{12}$, as that hydrocarbon is formed when the colouring matter is distilled with zinc-dust. In its tinctorial properties and absorption-spectrum, anchusin resembles quinizarin.

The most characteristic test for alkanet and alkannin is the absorption-spectrum. The solution in amyl alcohol gives the best results, and exhibits three equidistant bands in the blue-green. On adding ammonia these give place to two bands, one nearly coincident with and the other on the red side of the *D* line.

Alkanet root may be assayed by treating it with ether, which should extract not less than 5% of colouring matter. It is one of the most ancient dyestuffs and was formerly used for dyeing various shades of violet, lilac, and lavender, but has been superseded for such purposes. It is used for staining wood crimson, and is employed in perfumery for colouring oils and pomades. An alkaline solution is sometimes used to colour syrups. Tincture of alkanet forms a very good substitute for litmus.

SAFFLOWER

This consists of the dried florets of *Carthamus tinctorius* a plant resembling the thistle. It is indigenous to Egypt and the Levant, but is cultivated in other countries. It varies much in quality, the Egyptian being the richest in colouring matter, then the Indian and Chinese. The colour of good safflower is a fiery red; a dull red colour is an indication of bad preparation.

Safflower contains two yellow colouring matters, one of which is soluble in cold water, and exists in considerable proportion (20 to 30%); the other is insoluble in water, but dissolves in alkaline liquids. Beside these, safflower contains a small proportion of a red colouring matter, carthamin, which is the only constituent of value. The proportion of insoluble yellow colouring matter varies inversely with that of carthamin.

[1] Paper impregnated with an alcoholic solution of alkannin forms the so-called Boettger's test paper. It is very sensitive to free alkalies and alkaline salts and particularly to ammonia, the slightest traces of which colours the paper green.

Carthamin, $C_{25}H_{24}O_{12}$(?), the red colouring matter of safflower, forms only from 0.3 to 0.6% of the weight of the flowers. To prepare it, safflower is washed with cold water till no more soluble yellow colouring matter is removed, after which it is treated with water and 15% of its weight of crystallised sodium carbonate. The solution is strained from the insoluble portion, cotton yarn immersed in it, and the liquid acidified with citric acid. The cotton takes up the carthamin and an accompanying yellow colouring matter. When washed and treated with a weak solution of sodium carbonate, the carthamin dissolves,while the yellow dye remains fixed on the cotton. On acidifying the solution with tartaric acid, the carthamin is thrown down as a bright red, amorphous precipitate, which, when mixed with a little water, forms the *safflower extract* or *paste* of commerce.[1] The product may be further purified by solution in alcohol and precipitating with water.

Carthamin is insoluble in water or ether, but readily soluble in alcohol and in alkaline solutions. The cherry-red alcoholic solution dyes silk without a mordant, and, when allowed to evaporate on glass, leaves a varnish which appears red by transmitted light and a beautiful beetle-green by reflected light. On addition of an acid, the alcoholic solution of carthamin becomes yellow, and alkalies also turn it yellow or orange. The colouring matter is very unstable, and undergoes rapid alteration on exposure to air or when boiled with alcohol or water.

Carthamin has feeble acid characters. The ammonium salt yields, with stannic chloride, a yellowish-brown precipitate, with ferric chloride a brownish-red, and with mercuric chloride a red precipitate.

Carthamin dissolves with a red colour in strong sulphuric acid, probably forming a sulphonic acid, for the solution is not precipitated on addition of water.

Safflower is best assayed by a small dyeing test, and by an application of the method already described for detecting and separating any objectionable yellow colouring matter.

Safflower used to be employed for dyeing the "red tape" used for offices. Its use has much decreased of late years, but it is still employed to some extent to dye silk, cotton, and linen various shades of pink and red. On fabrics previously dyed with annatto it produces a scarlet.

[1] If the paste be triturated with French chalk and the mixture dried, a product is obtained which is employed as a *rouge*.

On the fibre, a rose, pink, or crimson colour due to safflower is immediately turned pale yellow by a single drop of alkali, and the colour is then destroyed by any further treatment. Weak acids do not affect the colour, but strong acids, chlorine, and sulphurous acid bleach it at once. Alcohol has no action, but ammonia changes safflower pink (on cotton) to a flesh-tint, and ammonium sulphide decolourises it.

The Colouring Matters in Flowers

The yellow, red, purple and blue pigments to which are due many of nature's most brilliant colour effects have naturally been frequently the subject of research, the literature being voluminous. An excellent systematic account will be found in Perkin and Everest's book on *The Natural Organic Colouring Matters*, pp. 235–344. Few of the flower pigments are useful sources of colour commercially but some are constitutionally allied to useful synthetic dyes.

Chlorophyl the green colouring matter in grass, leaves etc. has also been the subject of many researches. It contains two products, distinguished as chlorophyl *a* and chlorophyl *b* both of which contain nitrogen and are magnesium compounds. A good account of the chemistry of their substance appears in *Ber.*, 1917, **30**, 1777–1799.

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COLOURING SUBSTANCES IN FOODS

REVISED BY WALTER E. MATHEWSON

A wide variety of pigments, dyestuffs of animal and vegetable origin, and synthetic dyes has been employed for colouring foods.[1] As a class the colouring matters of fruits, vegetables and meat products are neither very brilliant nor permanent, so that artificial colouring materials equal to them in these respects and resembling them in hue are easily found. Inasmuch as the coal tar dyes possess the advantages of convenient solubility, cheapness, high colouring power and brilliancy, they have largely supplanted other colouring materials, excepting where thei remployment is restricted by legal enactments. The National laws of the United States, and of France which relate to the use of food colours may be taken as interesting examples of such administrative regulations.

The U. S. Food and Drug Act of 1906 prohibits the use of any poisonous colouring matter in food products coming within its jurisdiction. The Secretary of Agriculture (F. I. D. 184, July 10, 1922) has named the following coal tar dyes that, generally speaking, may be employed as food colours when not concealing damage or inferiority. The numbers preceding the names refer to the work by A. G. Green entitled "A Systematic Survey of the Organic Colouring Matters founded on the German of Schultz & Julius." London 1904. 56. Ponceau 3R, 107. Amaranth, 517. Erythrosine, 85. Orange I, 4. Naphthol Yellow S, 94. Tartrazine, Yellow A. B. (Benzene-azo-β-naphthylamine), Yellow O. B. (Ortho-tolune-azo-β-naphthylamine), 433. Guinea Green B, 435. Light Green S. F. Yellowish, 692. Indigo Disulphoacid.

[1] The chapter on food colours in the 4th edition of this work was written by A. F. Seeker. Further studies in the field have led to the introduction of some new methods and to the extension of many of the others, so that it seemed desirable to completely rewrite this portion of the volume. The excellent article in the 4th edition has been used freely however and in many cases where it has been impractical to give definite references. W. E. M.

Most common colouring matters of natural origin, such as those of annatto, turmeric, saffron, caramel, chlorophyll and cochineal, have been considered non-poisonous by the federal authorities.

The French law of June 28, 1921 (*Bull. International de la Repression des Fraudes* 1912, **206.** *Cf. Muttelet, Ann. falsifications,* 1911, **4**, 324) permits the use of the coal tar dyes named below for the colouring of liqueurs, preserved fruits, candies, meat casings, cheese coatings and certain other food products:

512 Eosine
517 Erythrosine
520 Rose Bengale
54 Scarlet R
55 Ponceau 2R
64 Crystal Ponceau
65 Bordeaux B
106 New Coccine
105 Fast Red E
107 Amaranth
462 Acid Fuchsine
85 Orange I
4 Naphthol Yellow S
84 Chrysoine
425 Auramine O (chloride)
427 Malachite Green (sulphate)
435 Light Green S. F. Yellowish
480 Water Blue 6B
440 Patent Blue
569 Indanthrene
451 Methyl Violet
468 Acid Violet 6B

Tartrazine and Flavazine S were tolerated as substitutes for Naphthol Yellow S from the latter part of the war period until 1923 (*Circular of the Minister of Agriculture, Ann. Falsifications* **15,** 1922, 250). Harmless vegetable colours, among which are included indigo, alizarine, and their sulphonated derivatives, caramel, annatto, chlorophyll and cochineal, may be used in foods of the classes just named, and also in ciders, vinegars, marmalades and edible oils and fats other than margarine. The use of the following inorganic pigments is permitted also in certain classes of food products: forms of ferric oxide, of manganese dioxide, of calcium carbonate and sulphate, Ultramarine, Prussian Blue, Cobalt Blue and Terre Verte (natural iron silicate). The use of copper salts for greening leguminous vegetables is allowed, provided the amount of copper present does not exceed 0.01%.

The comprehensive work of Hesse published as U. S. Dept. Agr. Bur. Chem. Bull. 147, discusses the use of coal tar dyes as food colours, presenting in concise form the results of scientific investigation, and recapitulating governmental rulings made in the U. S. and Europe prior to 1912.

The materials containing food colouring substances may be divided for convenience, into two classes: coloured foods, drugs and beverages; and commercial food colours.

Coloured food products may be examined to show the presence or absence of poisonous dyes and pigments, or simply to detect added colouring substances which conceal inferiority or make the products appear to be of better grade. The colour analysis is undertaken to disclose the nature of the tinctorial substances present, and this in most cases involves the removal of the bulk of the food material from the colouring matter, a treatment of the latter to separate it into its components if a mixture, and finally such further steps as may be necessary to identify the individual dyes or coloured compounds. Occasionally it is desirable also to estimate the colouring substances quantitatively. Analytical methods which are applicable for dealing with small amounts of substances must be used, as foods and beverages seldom contain more than 0.01% of added colouring matter, and the proportion is usually much less than this. The analysis of commercial food colouring materials is in most cases simpler, as the dyes are present in relatively large amounts, and the choice of methods available for identifying and estimating them is much less restricted. The analyst often has some information regarding the processes employed in manufacturing these products, and their examination may consist mainly in the estimation of the inert inorganic diluents and impurities, such as moisture, insoluble matter and salt.

QUALITATIVE EXAMINATION OF FOOD PRODUCTS FOR PIGMENTS

Pigments (other than the lakes of soluble colouring matters) are not very extensively employed as food colours. Among the commoner of these may be mentioned the following: Prussian Blue, Ultramarine, Indigo, Indanthrene; the various red, brown and yellow pigments, consisting essentially of oxides of iron but often containing manganese; carbonaceous blacks, such as lamp black and

bone black; and green copper compounds, especially the chlorophyll derivatives produced when green vegetables are boiled in copper vessels or with copper sulphate. The common pigments containing lead, chromium and other poisonous metals are seldom employed but instances of their use are occasionally reported by health officials.

Microscopical examination (See Winton, *Microscopy of Vegetable Foods*, New York, 1916), together with analyses of the ash of the product, will in most cases serve to identify pigment colours, since when these are used they are usually present in relatively large amounts. Pigments used in coatings often may be separated from the uncoloured portions of the food material by rinsing with water and allowing the washings to stand until the insoluble material has settled. Candies are conveniently dissolved in hot water and the mixtures centrifuged or allowed to settle. Granulated sugar containing blue pigments added to conceal a yellowish tint may be treated in a similar way, several hundred grm. of material being necessary to furnish sufficient pigment for the identification tests.

Ultramarine Blue and Ultramarine Violet are stable to alkalies, but are instantly decomposed and decolorised by dilute hydrochloric acid with liberation of hydrogen sulphide. The latter may be identified by its odour and effect on test papers moistened with solutions of lead acetate and ammoniacal cadmium chloride.

Carbon blacks must be identified by their physical properties and indifference to chemical reagents. They are easily distinguished from colouring matters such as unsulphonated nigrosine and benzidine blacks by their insolubility in hot alcohol, dichlorhydrine or phenol.

Lake pigments are insoluble derivatives of soluble colouring matters and usually are decomposed by treatment with hydrochloric acid and an organic solvent as described on page 441; and the treatment of food materials containing them is therefore identical with that described for the separation of soluble colouring matters in so far as the dye radicals are concerned. Microscopical examination will often show whether or not any dye substances present are in the form of lakes, but this information is seldom of interest except as an indication of the possible presence of objectionable heavy metals such as tin. Canned and preserved green vegetables, especially peas, are often coloured by cooking in copper kettles or by treating with a very dilute solution of copper sulphate. Willstaetter and

Stoll (*Untersuchungen ueber Chlophyll*, Berlin, 1913, p. 323) have shown that the products of acid hydrolysis of the chlorophylls react readily with copper compounds even at high dilutions producing stable derivatives of intense green colour. These metallic derivatives are soluble in organic solvents but the presence of the objectionable metal can be shown most easily by incinerating a few hundred grams of the product and estimating the copper in the ash.

Zinc is also used for fixing the green colour of canned peas (cf. Cribb, *Analyst*, 1925, **50**, 286).

The alumina and lime derivatives of cochineal and of yellow vegetable dye stuffs, especially Persian berries, are perhaps the commonest of lake colours used in food products. Tin lakes, especially that with cochineal, were formerly not uncommon. Lakes containing chromium, lead, antimony, barium and other poisonous metals are employed as painters colours and under exceptional circumstances may be used in foods. The ordinary methods of inorganic analysis are applicable for the estimation of the foreign metals, the organic matter being removed by incineration or by digestion with sulphuric and nitric acid.

A few of the red and orange coal tar dyes, such as Para Red, are almost insoluble, and when mixed with inert substances are employed as pigments. These colouring matters dissolve sufficiently in organic solvents so that they will be detected in the routine treatment for soluble dyes.

Read (*U. S. Treasury Decision* 32322: *Orig. Communications, 8th Intern. Congress App. Chem.* **18**, p. 301) uses the method given below for detecting Indigo and Prussian Blue in teas:

Place about 25–50 grm. of tea in a sieve having 40 to 60 meshes to the inch and shake over a piece of white paper. If the tea is tightly rolled it should be slightly crushed, either before putting it into the sieve or by rubbing it against the latter. Drag a spatula or the blade of a case knife flat side downward over the paper, crushing the dust between the blade and the paper. A little pressure applied by the finger to the end of the blade will be necessary to secure the proper crushing or streaking effect. Any particles of pigment in the dust will be revealed by a coloured streak on the white paper. If black unglazed paper be used, white facing pigments can be detected in the same way by the appearance of white streaks. A lens with a magnification of 8 to 12 diameters is useful in detecting the smaller

streaks. Observation of the streaks must be made in bright daylight, sunlight being desirable.

The character of the pigments present may be determined as follows: A black glossy streak indicates carbon. A blue streak may be due to Prussian Blue, Ultramarine or Indigo. Treat the colour either directly on the paper, or after transferring it to a microscopic slide, with a drop of 40% sodium hydroxide solution. Prussian Blue will become yellowish-brown; Indigo or Ultramarine will remain unchanged in colour. Treat another portion of the streak with 10% hydrochloric acid. The blue colour of Ultramarine is discharged; Indigo remains unchanged. A yellow streak may indicate turmeric. This becomes bright red upon treatment with a mixture composed of concentrated hydrochloric acid saturated with boric acid. Concentrated sulphuric acid also turns turmeric bright red.

Knight (*J. Ind. Eng. Chem.* 1914, **6**, 909) detects Prussian Blue by a test for cyanides.

QUALITATIVE EXAMINATION OF FOOD PRODUCTS FOR SOLUBLE COLOURING MATTERS

Direct Tests

Simple tests for the artificial soluble colouring matters are usually made before a more thorough examination is undertaken, and occasionally they furnish all necessary information. The commonest of the colouring matters naturally occurring in food products are those of the three groups; the yellow oil-soluble carrotinoids found in butter fat, egg yolk, yellow animal and vegetable edible oils, carrots, tomatoes and many yellow flowers; the glucoside anthocyans present in most red and purple fruits; and the brown soluble colouring matters, such as caramel, formed by heating sugars and similar substances. The colouring matters of these groups are discussed on the following pages in connection with the various tests and methods, but the properties mentioned below are most frequently applied for their differentiation from synthetic dyes.

The Carrotinoids are insoluble in water and dilute aqueous solutions of alkalies and acid. In mixtures of petroleum spirit with dilute alcohol they show high relative solubility in the petroleum spirit. They are rather unstable toward oxidising agents and

cannot be fixed easily on silk or wool. Caramel and the anthocyans are relatively much more soluble in water and in aqueous hydrochloric acid mixtures than in amyl alcohol. Caramel shows but little affinity for wool, and whilst the anthocyans stain the fibre somewhat more strongly, their dyeings are destroyed by alkalies.

Wool Dyeing Procedure

One of the most useful of the simpler methods for detecting artificial colouring matter is the so-called dyeing test. Arata (*Zeitsch. anal. Chem.* 1889, **28**, 639) found that the common coal tar dyes could be detected conveniently in wine by slightly acidifying the liquid and boiling with a small amount of white wool fibre, the latter becoming deeply coloured by any artificial dye present. It was shown by Winton (*J. Amer. Chem. Soc.*, 1900, **22**, 582) that a similar procedure may be followed with a large variety of food products, solid materials being first mixed to a paste with water. The method as applied to acid dyes was much improved by Sostegni and Carpentieri (*Zeitsch. anal. Chem.*, 1896, **35**, 397) who purified the artificial colouring matters by separating them from the dyed fibre with boiling dilute ammonia water, then fixing them again on a smaller piece of clean wool. The following description of the test is that given in *Tentative Methods of the Association of Official Agricultural Chemists*, 1919. (*Cf.* Seeker, this work, 4th Ed., volume V; Loomis *U. S. Dept. Agr. Bur. Chem. Circ.* 63; *U. S. Dept. Agr. Bull.* 448.)

(*a*) **Wines, Fruit Juices, Distilled Liquors, Flavouring Extracts, Vinegars, Beers, Syrups, Non-alcoholic Beverages and Similar Products.**—Dilute 20–200 c.c. of the sample with 1–3 volumes of water and boil or heat on the steam bath with a small piece of white woolen cloth (nun's veiling). When the mixture contains much alcohol, heat until most of the alcohol has been removed; in other cases, take out the wool after 5–15 minutes and rinse with water. Then treat the liquid with 3 or 4 drops of concentrated hydrochloric acid for each 100 c.c. and warm again for 10–20 minutes with a clean piece of wool. The basic dyes go on the fibre best from neutral solutions and, if present, will appear on the first piece of wool. Acid colours dye from neutral solutions, but more readily from those containing free acid. If the wool takes up any considerable amount of colouring matter in either case, the presence of coal tar dyes is indi-

cated. The lichen colours (Archil, Cudbear, Litmus) go readily on wool, however, and many other natural colours, such as Turmeric, will dye the fibre, if present in considerable amount. On the other hand, a few coal tar dyes, such as Indigo, Disulphonic acid, and especially Auramine O and Naphthol Green B, are unstable, and, if present in but small amounts, may give no distinct dyeing. Acid dyes are much more frequently used than basic dyes and in most cases may be removed from wool without much decomposition by "stripping" the fabric with dilute ammonia. By the action of the alkali, many natural colours are destroyed, whilst others remain for the most part on the fibre. If the behaviour with wool in neutral and acid solutions indicates the presence of acid dyes, rinse the coloured cloth thoroughly with water, cover with 2% ammonium hydroxide solution in a basin, boil for a few minutes, remove the cloth and squeeze out the adhering liquid. Boil the ammoniacal solution to remove the excess of ammonia, drop in a piece of clean, wet wool, make distinctly but not strongly acid with hydrochloric acid and boil again. If acid coal tar dyes are present, they will usually give a fairly clean, bright dyeing on the second piece of wool. A further purification may be carried out by repeating the stripping and re-dyeing, though generally accompanied by corresponding loss of dye.

(*b*) **Candies and Similar Coloured Sugar Products.**— Dissolve about 20 grm. of the sample in 100 c.c. of water and treat the solution as directed under (*a*). When the colouring matter is on the surface of the candy, pour off the solution before the colourless inner portion has been dissolved.

(*c*) **Jams and Jellies.**—Boil a mixture of 10–20 grm. of sample and 100 c.c. of water with wool in neutral and also in acid solution as directed under (*a*). For thick jams it is usually better, though less easy, first to extract the colouring substances by treating the product as directed under (*d*).

(*d*) **Canned and Preserved Fruits and Vegetables, Sausage Casings, Smoked Fish, Coffee, Spices, Etc.**—Macerate 20–200 grm. of the sample with 4–5 times its weight of 80% alcohol. After standing a few hours pour off the solvent as completely as possible and repeat the extraction, using 70% alcohol containing about 1% of ammonia. 1. Examine separately the filtered alcoholic extracts as directed under (*a*); or, 2. Boil the ammoniacal solution until

practically neutral, complete the neutralisation with acetic acid, add the neutral 80% alcohol extract, continue the evaporation until most of the alcohol is removed and boil with wool as directed under (*a*).

(*e*) **Cocoa and Chocolate Products.**—Treat cocoa as directed under (*d*). The alcoholic extract will contain a large amount of natural colouring matter, and several dyeings and strippings may be necessary to get rid of this in order to show the presence of coal tar dyes.

Chocolate may be treated similarly, but the following procedure is preferable: Wash 20–200 grm. of the well-divided sample with gasoline on a filter until most of the fat has been removed; if the gasoline is coloured, reserve for the examination for oil-soluble dyes as directed below. Remove most of the adherent solvent from the residue by evaporation, or pressure between layers of absorbent paper, and digest with alcohol as directed under (*d*).

Coal tar dyes may also be detected in chocolate and cocoa products by mixing samples directly with 3–4 times their weight of hot water and immediately boiling the magma with wool, as directed under (*a*). Because of the presence of large amounts of fatty and protein materials, this method is not very satisfactory.

(*f*) **Cereal Products.**—Proceed as directed under (*d*), in most cases working with a large amount of the sample, 200–500 grm., and a relatively smaller amount of alcohol. Where tests are to be made only for the acid dyes, the extraction with neutral 80% alcohol may be omitted advantageously.

Oils and Fats.—Prepare an alcoholic solution of the oil-soluble dye by one of the following methods, which are to be applied with oil or fat obtained by extraction with ether or gasoline, if the nature of the substances require it.

(*a*) Shake the oil or melted fat with an equal volume of 90% alcohol. The alcohol after separation will contain Aniline Yellow, Butter Yellow, Aminoazotoluene and Auramine, if present.

(*b*) Saponify 20–200 grm. of the oil or fat with alcoholic potassium hydroxide and, after removal of most of the alcohol on the steam bath, extract the soap with ether or gasoline. Most of the common dyes are removed by this treatment, though the digestion with strong alkali may cause some decompositon and make the extraction rather troublesome.

(*c*) Dilute 20–200 grm. of the oil or melted fat with 1–2 volumes of gasoline and shake out successively with 2–4% potassium or sodium hydroxide solution, 12–15% hydrochloric acid, and phosphoric-sulphuric acid mixture, prepared by mixing 85% phosphoric acid with about 10–20% by volume of concentrated sulphuric acid.

The dilute alkali extracts Sudan G and Annatto. The dilute hydrochloric acid extracts Aniline Yellow (7), Aminoazotoluene, and Butter Yellow (16), the first two forming orange-red, the latter cherry red solutions in this solvent. The phosphoric acid mixture is necessary for the extraction of Sudan I (II), Sudan II (49), Sudan III (143), and the homologue of the last, Sudan IV. Benzene-azo-β-naphthylamine and homologues also come in this group, though they readily undergo chemical changes in the strongly acid mixtures. The procedure is not very suitable in the presence of Auramine, but this dye is seldom found in oils. Neutralize the alkaline and dilute hydrochloric acid solutions; dilute the phosphoric acid mixture and partially neutralise, cooling the liquid during this operation; and extract the dyes by shaking with ether or gasoline.

For the direct dyeing test use the alcoholic solution obtained as directed in (*a*). Evaporate to dryness the ether or gasoline solutions, obtained as directed in (*b*) and (*c*), and dissolve the residue in 10–20 c.c. of strong alcohol. To the alcoholic solution add some strands of white silk and a little water and evaporate on the steam bath until the alcohol has been removed or until the dye has been taken up by the silk. The dyeing test is sometimes unsatisfactory, and in all cases a small portion of the alcoholic solution should be tested by treating with an equal volume of concentrated hydrochloric acid and stannous chloride solution. The common oil-soluble coal tar dyes are rendered more red or blue by the acid and are decolorised by the reducing agent. Most of the natural colouring matters become slightly paler with the acid and are little changed by the stannous chloride solution.

Basic colours may sometimes be stripped from the fibre with alcohol or strong acetic acid and again fixed on wool after boiling off or neutralising the solvent.

The wool dyeing test is well suited for the detection of water-soluble dyes, but it is not so convenient with oil soluble colours, and perhaps would seldom be used with the latter if it were not for the fact that some of the dyed fibre can be kept with the analyst's notes and exhibited as evidence in case of any controversy.

DIRECT METHODS FOR OIL-SOLUBLE COLOURS

Doolittle's test (*U. S. Dept. Agr. Bur. Chem. Bull.* 65, p. 152), which is quickly made, consists in shaking a few c.c. of the oil or melted and filtered fat, in a test tube with an equal volume of dilute hydrochloric acid and a little ether. A second portion of the material is treated in a similar way with dilute sodium hydroxide. An orange or reddish coloration in the acid indicates a coal tar dye, a yellowish or brownish coloration in the alkali, annatto. Most of the common oil-soluble azo dyes can be detected by this test but it is not very satisfactory for yellow A. B. and yellow O. B.

Cornelison (*J. Amer. Chem. Soc.*, 1908, **30**, 1478) uses glacial acetic acid for removing the colouring matter from fat, his method which is quite widely applicable, being as follows:

About 10 grm. of the clear dry fat are melted and well shaken in a separatory funnel with 10–20 grm. of glacial acetic acid (99.5%). If the materials are too hot the fat will dissolve, but at about 35° it separates quickly and almost completely. The clear acid is drawn off, and after noting its colour, it is tested by adding to 1 portion of 5 c.c. a few drops of concentrated nitric acid and to another portion a few drops of conc. sulphuric acid. The reactions are indicated in the following table:

Colouring matter	Colour of acid extract	Conc. HNO_3	Conc. H_2SO_4	H_2SO_4 and enough ether to clear solution
Pure natural butter (yellow).	Colourless.	Colourless.	Faint pink after a while.	Colourless.
Soudan I.	Decided pink.	Strong pink.	Strong clear pink.	Pink.
Butter Yellow	Very faint pink.	Faint pink.	Faint pink.	Faint colour.
Cerasin Orange G. (Cassella).	Greenish-yellow, strong.	Acid yellow, oil globule salmon-pink.	Same as with HNO_3.	Brownish-yellow.
Yellow O. B. (H. & M.)	Bright yellow, not very strong.	Acid faint pink, oil globule salmon-pink.	Similar to HNO_3.	Pink.
Yellow A. B. (H. & M.	Warm ochre-yellow, weak.	Pink, fat colourless.	Brownish-pink, oil faint pink.	Pink.
Annatto..........	Dull yellow.	Little change.	Faint pink after a while.	Very faint yellow.
Curcumin.........	Intense greenish-yellow.	Dull ochre-yellow.	Strong pink.	Yellow.
Carrot..........	Very faint greenish yellow.	Faint yellow.	Faint pink after a while.	Very faint yellowish.
"Alderney butter colour" (H. & M.)	Brownish-yellow.	Strong pink.	Strong pink.	
Ranson's butter colour ("Vegetable").	Yellow.	Almost decolorised.	Same as HNO_3.	....
"Dandelion" brand butter colour ("Vegetable").	Yellow.	Almost decolorised.	Same as HNO_3.	....

Gilmour (*Analyst*, 1920, **45**, 173), detects artificial colouring matter in butter as follows: One c.c. of the clear filtered fat (which must be separated at a temperature not above 100°) is placed in a test tube and immersed in an oil bath which is raised to about 185°. The test tube is removed occasionally, shaken and replaced. In absence of coal tar dyes the fat will become colourless in ten minutes.

Other methods for fats and oils are those of Martin (*Analyst*, **1**, 70). Geisler (*J. Amer. Chem. Soc.* 1898, **20**, 110). Palmer and Thrun (*J. Ind. Eng. Chem.* 1916, **8**, 614) and Lubs (*J. Ind. Eng. Chem.* 1918, **10**, 436). See also page 493.

Tests for Caramel

Amthor (*Zeitsch. anal. Chem.*, 1885, **24**, 30) tests for caramel as follows: 10 c.c. of the liquid are placed in a tall cylindrical vessel and treated with 30–50 c.c. of paraldehyde. Absolute alcohol is added in small portions, with vigorous shaking after each addition, until the liquids mix. If caramel is present a brownish precipitate will settle out, depending in colour upon the amount of caramel present. The supernatant liquid is decanted and the precipitate washed twice with absolute alcohol, after which the residue is dissolved in a small amount of hot water and filtered. The colour of this solution will give some idea of the amount of caramel present. The colour may be identified by treating this filtrate with an equal volume of freshly prepared phenylhydrazine reagent (2 parts phenylhydrazine hydrochloride, 3 parts sodium acetate, and 20 parts water) which in the presence of considerable caramel gives a dark brown precipitate in the cold, the reaction being hastened by warming. When small amounts are present the precipitate sometimes takes about 12 hours to collect.

Crampton and Simons (*J. Amer. Chem. Soc.*, 1899, **21**, 355) treat 50 c.c. of the liquid with 25 grm. of fuller's earth, shake vigorously, leave for half an hour and filter. The colour of the filtrate should be compared in a tintometer with that of the original liquid and the percentage of colour removed by the treatment noted, this furnishing some indication of the proportion of colour due to caramel. All grades of fuller's earth do not absorb caramel sufficiently to be employed in this test, and the variety at hand should always be tested by control experiments before being used. The results obtained by this test should be accepted with caution, as the natural colour

of some substances like vinegar (*J. Amer. Chem. Soc.*, 1907, **29**, 75) are at times largely removed by fuller's earth, and unless the liquid is almost decolorised (80% or more removed) it is not safe to conclude that caramel is present without confirmatory tests by another method.

Fradiss (*Z. Zuckerind.*, 1899, **28**, 229) tests for caramel in dry substances by extracting with warm, pure methyl alcohol. The brown solution is filtered, and chloroform or amyl alcohol added to the filtrate, which causes a brown flocculent precipitate to form.

Woodman and Newhall (*Mass. Inst. Tech. Quarterly*, 1908, **21**, 280) found that in applying the Amthor test directly to vanilla extracts containing sugar the results are obscured by precipitation of the latter, together with some of the natural colouring matter. This is true of many other preparations, and their method of employing a preliminary treatment with zinc hydroxide is subject to fairly general application. They recommend that 15 c.c. of vanilla extract be mixed with 2 c.c. of zinc chloride (5% solution) and 2 c.c. of potassium hydroxide (2% solution). The precipitate is filtered off, washed with hot water to remove sugar, and then dissolved in 15 c.c. of acetic acid (10% solution). This is concentrated to about half its volume, the excess of acid neutralised, and the solution divided between 2 test tubes. To one of these 3 volumes of paraldehyde are added and just sufficient alcohol to make the mixture homogeneous. To the other is added an equal volume of freshly prepared phenylhydrazine reagent (see Amthor test immediately above). If caramel is present, both tubes will exhibit a brown precipitate on standing over night.

The following method for the detection of caramel and other common colouring matters in milk is given by Leach (Food Inspection and Analysis 4th edition revised and enlarged by A. L. Winton, New York, 1920, p. 160).

About 150 c.c. of the milk are curdled by the aid of heat and acetic acid, preferably in a porcelain casserole over a Bunsen flame. By the aid of a stirring-rod the curd can nearly always be gathered into one mass, which is much the easiest method of separation, the whey being simply poured off. If, however, the curd is too finely divided in the whey, the separation is effected by straining through a sieve or colander. All of the Annatto, or of the coal-tar dye present in the milk treated would be found in the curd, and part of the

caramel. The curd, pressed free from adhering liquid, is picked apart, if necessary, and shaken with ether in a corked flask, in which it is allowed to soak for several hours, or until the fat has been extracted, and with it the Annatto. If the milk is uncoloured, or has been coloured with Annatto, on pouring off the ether the curd should be left perfectly white. If, on the other hand, Aniline Orange or Caramel has been used, after pouring off the ether the curd will be coloured more or less deeply, depending on the amount of colour employed. In other words, of the three colours, Annatto, Caramel, and Aniline Orange, the Annatto only is extracted by ether. If Caramel has been used, the curd will have a brown colour at this stage; if Aniline Orange, the colour of the curd will be more or less bright.

Tests for Annatto.—The ether extract, containing the fat and the Annatto, if present, is evaporated on the water bath, the residue is made alkaline with sodium hydroxide, and poured upon a small, wet filter, which will hold back the fat, and, as the filtrate passes through, will allow the Annatto, if present, to permeate the pores of the filter. On washing off the fat gently under the water-tap, all the Annatto of the milk used for the test will be found to have been concentrated on the filter, giving it an orange colour, tolerably permanent and varying in depth with the amount of Annatto present. As a confirmatory test for Annatto, stannous chloride may afterwards be applied to the coloured filter, producing the characteristic pink colour.

Tests for Caramel.—The fat-freed curd, if coloured after the ether has been poured off, is examined further for Caramel by placing a portion of the curd in a test-tube, and shaking vigorously with concentrated hydrochloric acid. If the colour be Caramel, the acid solution of the coloured curd will gradually turn a deep blue on shaking, as would also the white fat-free curd of an uncoloured milk, the blue coloration being formed in a very few minutes, if the fat has been thoroughly extracted from the curd; indeed, it seems to be absolutely essential for the prompt formation of the blue colour in the acid solution that the curd be free from fat. Gentle heat will hasten the reaction. It should be noted that it is only when the blue coloration of the acid occurs in connection with a coloured curd that Caramel is to be suspected, and if much Caramel be present, the coloration of the acid solution will be a brownish blue. If the above

treatment indicates Caramel, it would be well to confirm its presence, by testing a separate portion of the milk in the following manner. (See *Nineteenth Annual Report of the Mass. State Board of Health* (1887), p. 183.)

About 4 oz. of the milk are curdled by adding to it as much strong alcohol. The whey is filtered off, and a small quantity of subacetate of lead is added to it. The precipitate thus produced is collected upon a small filter, which is then dried in a place free from hydrogen sulphide. A pure milk thus treated yields upon the filter-paper a residue which is either wholly white, or at most of a pale straw colour, while in the presence of Carmel, the residue is a more or less dark-brown colour, according to the amount of Caramel used.

Jagerschmid (*Zeitsch. Nahr. Genussm.*, 1909. **17**, 269) proposes the use of a modification of Fiehe's artificial honey test in the detection of Caramel.

Preliminary Spectroscopic Examination

The spectroscope is occasionally useful for the direct examination of food products, but as the absorption bands of most common colouring matters are not sharply defined, it is usually difficult to determine the position of maximum absorption with such precision as to differentiate dyes of similar hue. Furthermore, it is only in exceptional cases that there is ground for the assumption that but a single colouring matter is present, and in mixtures the superimposed absorptions produce maxima that in most cases differ more or less in position from those shown by the component dyes alone. The colouring matters named on page 463 give unusually characteristic spectra and hence may sometimes be identified directly in liquid food materials in this way.

General Methods for the Qualitative Separation or Isolation of Soluble Colouring Matters

In the United States, where but a limited number of artificial colouring matters are permitted by law, it is necessary to employ mixtures to obtain intermediate hues. However, the colouring of food products with dye mixtures is widely practiced in all civilised countries.

Water-soluble colouring matters may be separated from food products and from each other by the wool dyeing method; by direct treatment of the acidified product with amyl alcohol; and by the general method of extracting the colour from any solid materials present and treating the solutions so obtained with immiscible solvents to effect the separation of the individual colouring matters from each other and from soluble colourless substances.

Separation of Dyes by Wool Dyeing Method

The separation from the food material with wool fibre has already been described on pages 431 *et seq.*

The wool dyeing procedure as a method for separating dyes from food substances is especially indicated; 1st, when as with cocoa products the material contains large amounts of natural colouring matters as normal components, and the chief object of the examination is to detect added dyes. In such cases a second stripping and re-dyeing may be of advantage. 2nd, when it is desired to separate highly sulphonated dyes containing reactive amino groups, as for example, the Fast Yellows No. 8 and No. 9. If these dyes are separated by the use of amyl alcohol and strong hydrochloric acid they are somewhat altered, presumably through the combined action of the strong acid and impurities in the solvents, and they can no longer be diazotized and coupled (see *J. Ind. Eng. Chem.*, 1920, **12**, 883).

The dyeing test may be applied to the detection and separation of mixtures of dyes, as these differ in the ease with which they become fixed on wool. Seeker (*This work, 4th Ed. Volume V*, p. 554) boils several small pieces of wool separately in the same bath, allowing each piece to remain in it for about one minute, and preserving the order in which they were used. Mixed dyes will be indicated by a gradual change in the colour of the fibre from the first to the last. By separating the pieces and selecting those containing the predominating amounts of the various shades, stripping, and re-dyeing in the same manner, a fairly satisfactory separation of the colours can often be obtained.

The mixture of Naphthol Yellow S and Ponceau 2R (or 3R), frequently used for tinting macaroni products, may be dyed on wool, and the wool stripped first with lukewarm, then with boiling ammonia water. At low temperatures the Ponceau is extracted much less readily than the Naphthol Yellow S.

Separation of Dyes with Amyl Alcohol and Hydrochloric Acid

Direct treatment of the strongly acidified products with amyl alcohol is especially useful for the separation of soluble acid colouring matters used in the form of lakes, especially those of the natural yellow dye stuffs, Persian Berries and Fustic. The method is usually convenient and satisfactory for the routine examination of liqueurs, carbonated beverages, syrups and confections when these products are moderately deeply coloured in yellow, brown, orange or red hues.

Liquid or pasty foods are treated with one third volume of concentrated hydrochloric acid and one third to two thirds volume of amyl alcohol, shaken vigorously and centrifuged or allowed to settle. Solid materials, such as macaroni, are well ground, the powder mixed with amyl alcohol, concentrated, or 1:1 hydrochloric acid added, and the mixture allowed to stand for a time with occasional shaking. Wines, liqueurs and similar beverages are diluted and evaporated somewhat on the steam bath to remove alcohol before the treatment with acid and organic solvents. Candies and similar soluble materials are dissolved in three or four parts of hot water, the solutions then treated with one third volume of concentrated hydrochloric acid and shaken with the solvent, as described above. The amyl alcohol extract obtained by this procedure is removed by decantation or by means of a separatory funnel, and the material re-extracted with a second (smaller) amount of the solvent, when it seems desirable to do so to obtain sufficient colouring matter for subsequent tests. The combined amyl alcohol extract containing the colouring matter may be washed with successive portions of hydrochloric acid of decreasing acidity, or more simply with successive portions of one half its volume of water, these being drawn off into separate test tubes or beakers. Because of the acid dissolved in the amyl alcohol these washings will show a gradual decrease in acidity, and the colouring matters will appear in *maximum* amount in the different fractions according to their respective solubilities. Ponceau 6R (108) is washed out chiefly while the acidity is still high, N/1 or above. Amaranth (107), Brillant Scarlet (106) and Tartrazine (94) appear when the washings have an acidity between N/1 and N/4; Orange G (14) and Soluble Blue (480) between N/2 and N/16; Palatine Scarlet (53), Ponceau 2R (55) and 3R (56), Naphthol Yellow S (4), Cochineal (706), Crystal Ponceau (64) and Azorubien

A (103) between N/16 and N/256. When the acid is practically all removed, Orange I (85), Orange II (86) and Croceine Orange (13) begin to wash out, and less readily, Orange IV (88) and Metanil Yellow (95). Finally the unsulphonated colouring matters, such as Erythrosine G (156), Erythrosine (517) and the Rose Bengales (520 and 523), are removed by water very slowly or not at all when all traces of acid have been removed.

It must be remembered that a progressive change in the hue of the different washings or fractions does not always indicate the presence of more than one dye, as it may be due only to the higher acidity of the first washings. Whether or not this is the case can be ascertained by treating small test portions of the different washings with a slight excess of sodium acid carbonate, which will bring them to the same hue if but one colouring matter be present.

Amyl alcohol shows much less tendency to form obstinate emulsions with strongly acid than with neutral mixtures, and in this method the solid and colloidal portions of the food material are removed from the solvent while much acid is still present. However, troublesome emulsions are obtained occasionally that can scarcely be broken without a good centrifuge. Warming the mixtures tends to separate the solvents, but should be avoided in so far as possible because of the action of the hot acid on sugar, unstable colouring matters, etc.

Ordinarily in carrying out this procedure the maximum quantity of any Indigo Disulphoacid present will appear in the second and third washing, Amaranth and Tartrazine about the third washing, Ponceau 3R in the fourth or the fifth, Naphthol Yellow S in the fifth or sixth. The seventh washing is usually nearly neutral, and if the solvent is mixed with an equal volume of gasoline and again washed any Orange 1 present will be taken out almost completely. Erythrosine will remain dissolved in the mixture of solvents (unless the wash water contains a trace of alkali) forming a colourless or very faintly brownish solution of the free colour acid which, however, on shaking with a little dilute alkali becomes rose coloured, the dye passing into the aqueous layer.

Although the three common dyes Indigo Carmine No. 692, Tartrazine No. 94 and Amaranth No. 107, which tend to appear in the same washings, can be separated by combining the solutions containing them and refractionating with suitable solvents (page 461), it is

simpler to separate them by chemical means when ether dyes appear to be absent. If a hot solution containing the three colouring matters be treated carefully with a few small particles of sodium hydrosulphite (avoiding excess) until decolorised, the azo dyes are destroyed, whilst the blue is re-formed on shaking the hot liquid with air. Indigo Carmine may be destroyed by adding a few mg. of sodium nitrite to the hot acid solution and boiling for a few minutes. The azo dyes are scarcely affected, but will be further protected from the oxidising agent by adding a few crystals of urea before the nitrite. Tartrazine may be separated from Amaranth by treating the solution carefully with sodium hydrosulphite, which reduces the red dye first. The salt is added very slowly, a few small particles at a time, the mixture being well stirred meanwhile. When the colour changes to yellow the liquid is at once shaken with air to destroy any excess of hydrosulphite.

The dyes Ponceau 3R No. 56 and Naphthol Yellow S No. 4 tend to appear in the same washings, the maximum amount of the Ponceau[1] being often in the fifth and sixth portions, that of the Yellow in the sixth. As the latter dye is practically colourless in acid dilute solution, it is easily overlooked unless the washings are tested with alkali before being discarded. The two dyes are most readily separated by treating the washed portion containing them with one sixth volume of concentrated hydrochloric acid and shaking out with ethyl acetate. The ester when washed with sodium carbonate or ammonia solution (used in sufficient quantity to give an alkaline reaction) will become bright yellow, the dye passing into the aqueous layer.

The solubility data given on page 464 show that in the acid amyl alcohol treatment the strongly sulphonated triphenylmethane dyes (Light Green S. F. Yellowish, Acid Magenta, etc.) are extracted only in very small proportions. The extraction of the Acid Yellows and of Indigo Carmine is quite incomplete, but the last-named dye is usually taken up in such quantity as to permit of its ready identification in the first washings.

Guinea Green B and the similar dyes named on page 472 do not behave like the sulphonated azo colours in this procedure, as they are relatively more soluble in strongly acid than in nearly neutral aqueous solutions. When such colours are suspected they may be

[1] Neutral solutions of Ponceau 3R give with barium chloride solution a bluish red flocculent precipitate of the barium salt, even at rather high dilutions. Ponceau 2R gives a carmine red precipitate.

removed with dichlorhydrin (or phenol) mixtures, if the more general method of separation is not employed. Most of these dyes are green, blue or purple in hue.

Ammoniacal Cochineal or liquid Carmine, which is largely used as a food colour is decomposed by the acid treatment, the red solution becoming orange within a few minutes after the addition of the acid. The carminamide is hydrolysed with the formation of ordinary carminic acid, the latter being readily extracted by amyl alcohol and coming out chiefly when the acid normality of the washings is from $1/16$ to $1/256$. The change in relative solubility with change in acidity is somewhat less abrupt with carminic acid than with the common sulphonated dyes, so that the animal colouring matter shows less tendency to come out almost completely in a few fractions. When an orange colouring matter washes out between the third and eighth (or neutral) fractions it is advisable to test a few c.c. of the washings with acid stannous chloride solution or sodium hydrosulphite, which will at once decolorise the azo dyes but leave carminic acid unaffected. If this test and the appearance of the washings indicate that carminic acid only is present, the fractions may be combined, and, if not decidedly acid already, treated with a little more hydrochloric acid and finally shaken with a fresh portion of amyl alcohol which will extract almost all of the dye. The acid layer is drawn off, some gasoline added to the amyl alcohol and the mixture shaken with a little water to take out the colouring matter. In this way the colouring matter is quickly obtained in a fairly concentrated solution, and a portion of it can be tested by treating with a few drops of a 5% solution of uranium acetate, then carefully adding sodium acetate solution until the free hydrochloric acid is just neutralised, when the characteristic green uranium lake will be obtained. The mineral acid when not present in large excess may also be neutralised by cautiously adding the uranium acetate solution only, until the green coloration appears. The colour of the original mixture and its behaviour on treatment of acid usually show conclusively whether it contains ordinary Cochineal or liquid Carmine.

When an orange coloured dye washes out at low acidity it is often advisable to test a few drops with ammonium hydroxide before proceeding further, a purple coloration usually indicating Cochineal, Orange 1, Archil or Alizarine Red.

GENERAL METHOD FOR THE SEPARATION OF COLOURING MATTERS WITH IMMISCIBLE SOLVENTS[1]

The material is first treated with some solvent that will bring the colouring matters into solution. Commercial food colours, if soluble, are dissolved directly in water, care being taken that the solution be not made too concentrated. Solutions of suitable concentration contain from 0.05 to 0.01% actual colouring matter.

Candies, syrups, and other sugar products are taken up directly with hot water.

Wines, liquors, and other alcoholic beverages may be first diluted and warmed on the water bath to remove alcohol. However, for the subsequent treatment with immiscible solvents it is not necessary that the alcohol be driven off, as it is sufficient to dilute the sample to reduce the alcohol content to 10% or less. Sometimes this is preferable to heating the sample; but it must be borne in mind that the distribution of the colouring matters will be more or less modified by the alcohol.

Solid materials, such as fruits, flesh foods, cheese coatings, etc., may be extracted first with 80% alcohol, containing a very little acetic acid, to remove basic dyes, Cochineal, etc., sensitive to alkalies. The pulp separated from the acid alcohol solution may then be digested with dilute alcohol of from 65 to 80% strength, containing from 3 to 5% of ammonia. If both extracts are coloured it is easiest not to work them up separately, but to boil off the alcohol and ammonia from the second portion, and the alcohol from the first, and then combine them. A preliminary treatment with petroleum spirit as described on page 433, is often advisable with foods containing much fatty matter. If the petroleum spirit solution is coloured, it is reserved and examined for oil soluble dyes by shaking out with sulphuric acid as described below.

Wheat and rye products offer some difficulty in the extraction with dilute alcohol because of the solubility of the plant proteins, gliadin and hordein. The acid azo dyes with which macaroni, spaghetti, etc., are usually coloured may be separated with ammoniacal 70% alcohol as just described, it being necessary in almost all cases to use several hundred grm. of the food material. Boil the ammoniacal alcoholic extract containing the colouring matter until most, but not quite all, of the alcohol is removed. If the hot residue is of a semi-

[1] U. S. Dept. Agr. Bull. 448; see also Loomis, U. S. Dept. Agr. Bur. Chem. Circ. 63, Seeker, this work, Fourth Edition.

solid consistence, it is best to add a little alcohol. It is then treated with about one-half of its volume of concentrated hydrochloric acid and is poured into a large separating funnel. Amyl alcohol equal to about two-thirds of the original volume of the solution is added and sufficient salt solution to make the mixture separate well. After shaking, the amyl alcohol containing the colour is washed a few times with a salt solution containing hydrochloric acid to remove the protein; one separation with the centrifuge usually being desirable to free the solvent from coagulum. The solution so obtained may be treated further by the method described on pages 452 *et seq.*

Many of the common dyes may be separated from milk and ice cream by mixing these with several volumes of strong alcohol, filtering from the precipitated casein, and evaporating most of the alcohol from the filtrate. The solution thus obtained is nearly free from proteins, and the dissolved dyes usually can be separated satisfactorily by the further treatment with immiscible solvents.

The author's experience would indicate that alcohol is by far the most useful of the solvents available for the preliminary removal of dye from food materials. Phenol and aniline are excellent solvents, but expensive and troublesome to use. Aniline does not dissolve the wheat proteins.

Dissolved colouring matters are generally separated from fats and oils by saponifying the fat or oil with alcoholic potassium hydroxide and extracting the colouring matter from the soap with gasoline or ether. (See Gruenhut, *Chem. Zentr.*, 1898, **69**, II, 943.) The manipulation of this process is not very convenient and, of course, all unsaponifiable matter remains with the colour. It may be combined with advantage in many cases with one of the extraction methods with an immiscible solvent described below. A number of extraction methods are in use, and probably each possesses advantages for certain colours. Some dyes, as Aniline Yellow and Auramine, may be extracted from oils conveniently with 90% alcohol. (Frehse *Ann. Falsifications*, 1910, **3**, 293. Loomis, *U. S. Dept. Agr. Bur. Chem. Circ.* 63.) Cornelison's method (page 435) will serve for the extraction of almost all the common oil-soluble dyes. Much oil dissolves in the acetic acid, and a systematic fractionation is necessary, the different portions of extract being washed successively in several funnels containing a little gasoline.

If the oil or melted fat is diluted with 4 volumes of petroleum spirit, and the solution extracted with a mixture of 1 part water and 9 parts phenol, the colouring matters may be extracted, and by a fractionation procedure obtained in phenol solution fairly free from fatty material. By treatment with strong potassium hydroxide solution the phenol may then be dissolved and the dye taken up with ether. The action of strong acids on the colouring matter is avoided, but the manipulation is quite unpleasant.

The common oil-soluble dyes may be extracted from petroleum spirit mixtures by means of 25N sulphuric acid (page 435) or mixtures of sulphuric and phosphoric acids (page 493). The oil or fat is dissolved in 3 or 4 volumes of gasoline and shaken out with the acid, which effects a very complete separation in most cases. When Yellow A. B. or Yellow O. B. are separated by this process a large amount of the dye is lost through condensation reactions, which readily take place in the strongly acid mixtures. (*J. Ind. Eng. Chem.*, 1920, **12**, 883.) The loss can be minimised by adding other reactive substances, such as hydrazine sulphate or sulphanilic acid, to the acid, these being insoluble in petroleum spirit under all conditions and hence remaining in the acid after the latter is diluted, and the separated dye taken up with the organic solvent, an operation which in all cases should be carried out without delay. The petroleum spirit used must be purified, the following process giving a good product: 2000 c.c. of the petroleum spirit or low boiling point gasoline are treated with about 1 grm. powdered Yellow A. B. (Benzene-azo-β-naphthylamine) then shaken out with four or five 200 c.c. portions of sulphuric acid, the liquids being allowed to stand together for some time. An ordinary 5-pint acid bottle may be used for the mixing, the wash acid being removed or blown out with a wash bottle fitting.

The alkali salts of Sudan G and of the colouring matter of Annatto are readily soluble in water; hence these dyes are most easily removed by shaking out the solution of the oil or fat in petroleum spirit with dilute sodium or potassium hydroxide solution.

The oil-soluble dyes after their removal from the fatty matter will be obtained in solution in purified petroleum spirit or in some solvent from which they may readily be transferred to it by neutralization and re-extraction. The separation of the components can then be made by the fractionation process indicated on pages 448–499, employing

the data on pages 481–476. See also page 459, sections 15, 16 and 17.

Water-soluble colouring matters should be obtained by suitable preliminary treatment in aqueous or dilute alcoholic solution nearly free from acids, alkalies, or large quantities of salts. The alcohol content of the solution should not exceed 10%. Usually it is better to remove excessive alcohol (by evaporation) than to add water; but if the liquid contains so much sugar as to be syrupy it should be diluted. If the evaporation causes a separation of colouring substance, the sediment should not be removed before the treatment with immiscible solvents. When the colour has been extracted directly from solid products by acid amyl alcohol, this may be shaken out with salt solution, dilute hydrochloric acid, or water, as directed for the corresponding solution obtained in the first step of the procedure described on pages 451 *et seq.*, section 1.

Since the colouring substances of flowers and fruits are, generally speaking, rather unstable, especially in the presence of alkalies, it is well to divide the solution, one portion being examined especially for the coal tar dyes, the other reserved for additional tests for the natural colours. When coal tar dyes are not known to be present the wool dyeing test (page 431) should be made.

The analyst usually knows something in regard to the colouring matters present in a dye solution before beginning the systematic analysis. The best proceedure to be followed will depend upon what dyes are probably present; and no set method can equal in value a table of relative solubilities by means of which the distribution constants of any given dyes may be compared. It is, of course, advantageous in many cases to make group tests with small portions of the mixture, thus avoiding unnecessary and undesirable additions of reagents to the main solution.

In carrying out the fractionations described on pages 451 *et seq.* any given colour will, in general, appear in several washings, but where the maximum amount comes out will be evident from the solubility data; it being always remembered that these statements apply to solutions of concentration in the neighborhood of 0.01%, and that at widely different dilutions some variation may be expected. The solubilities of the components of the dye mixture are not likely to be so different as to allow even a qualitative separation by a single shaking out. It is usually necessary to employ more or less systematic

fractionation methods. For example, suppose a mixture is to be separated, of which it is known that one dye, when its amyl alcohol solution is shaken with an equal volume of acid of a certain concentration, distributes itself in equal amount between the two layers, whilst 94.1 per cent. of the other colour, under the same conditions, remains in the aqueous layer. If such a mixture in water solution is brought to the given acidity and is shaken out successively with three portions of amyl alcohol, each equal to one-fourth its volume, calculation shows that if the distribution ratios remain constant there will be present in the acid solution after the third shaking twenty-seven sixty-fourths or 42% of the first dye, one sixty-fourth or 1.6% of the second. Conversely, the first amyl alcohol portion, after two washings with portions of acid of concentration similar to that of the original solution, will contain 42% and 1.6% of the second and first dyes, respectively. Obviously, for a practicable quantitative separation, somewhat greater difference in solubility must exist; but it is usually sufficient to separate, in fairly pure condition, a portion of each of the colours that seem to be present, in order to characterise completely the component parts of the mixture. (For proceedure and calculation as to quantitative fractionations, no colouring matter being rejected, see pages 499–500.)

Emulsions, which occasionally cause trouble when working with impure mixtures, are most effectively broken by a good centrifuge. Should a solid stratum form between the two layers, it should be broken with a glass rod, the tube replaced in the centrifuge and whirled again. Heating tends to promote rapid separation, but the relative solubilities vary somewhat in hot mixtures. Strong acid solutions show much less tendency to emulsify than neutral or alkaline ones.

The final separation of mixtures of dyes of rather similar solubility will usually be made by selecting some pair of solvents in which they show a decided difference. Mixtures of dyes of practically identical solubility can, in most cases, be separated satisfactorily by chemical means or by precipitation reactions. Since the fractionation will have removed all except a few dyes belonging to a known group, suitable chemical methods may usually be chosen without difficulty.

The scheme described is not intended to be applied to relatively concentrated solutions. In practice, in the examination of coloured

food products, concentrated solutions are seldom or never obtained. The chief concern of the analyst here will be to avoid, as far as possible, the dilution of the colour by the use of unnecessarily large portions of organic solvents and washing liquids. Only in working with products sold for use as colouring matters, are solutions likely to be made too concentrated to be adapted to the scheme of separation.

As the common food dyes are, for the most part, salts of polybasic acids, the equilibrium conditions are obviously quite complex and concern not only the relative solubilities and dissociation constants of the free colour acid, but of the various acid salts and the sodium salt as well. Many dyes exist in more or less associated condition in ordinary solutions. However, it is found in practice that in most cases the distribution ratios with given acidity do not vary so greatly, but that fair results can be obtained on the assumption that they will remain constant. (See Reinders and C. Sely, *Zeit. Chem. und Ind. d. Koll.*, 1913, **13**, 96.)

Many of the acid and basic triphenylmethane dyes rearrange themselves into the tautomeric forms rather slowly when treated with alkalies, and their complete separation from such solutions by means of solvents is less simple than that of most other classes.

It will be noted from the solubility table, pages 464–468, that amyl alcohol, amyl alcohol and gasoline mixtures, and ether, although differing greatly in their power as solvents, show a sort of general correspondence in properties. They are especially suited for fractionations of such dyes as the sulphonated phenolic compounds, the distribution ratios of these changing greatly with varying hydrogen-ion concentrations. Dichlorhydrin, because of its solubility and non-volatility, is not very convenient as a solvent; nevertheless, it is almost indispensable for the separation of many colours. Aniline is an excellent solvent, but usually must be completely removed from a colour solution before tests are made, and will be employed only for a few separations. Both aniline and dichlorhydrin are conveniently removed from water solutions by shaking out with carbon tetrachloride. Phenol, like dichlorhydrin, shows a special solvent action toward the sulphonated triphenylmethane dyes and would be very useful for dye separations were it not for its disagreeable odour and effect on the skin.

The writer usually prefers to begin the treatment with immiscible solvents by shaking out with amyl alcohol from the neutral solution after addition of some sodium chloride. The outline which follows will indicate approximately the order in which the solvents will be chosen for a complex mixture.

The solution of the colouring matter, as free as possible from suspended matter, is treated carefully with sodium carbonate if it contains free mineral acid, or with acetic acid if it is alkaline. It should finally be neutral or very faintly acid. It should not contain the colouring matter in too great concentration, although when working with extracts of food products this latter condition is seldom encountered. Concentrations of about 0.01% may be taken as most suitable in general, and only in exceptional cases would stronger solutions (0.1%, for instance) be chosen by preference. In regard to the presence of alcohol, see page 445. When large amounts of dissolved foreign material, such as sugar, glycerol, etc., are present, it must be remembered that the solubilities of the colouring matters will be somewhat affected.

Since almost all colouring matters are found accompanied by small amounts of similarly coloured substances of different solubilities (subsidiary dyes, etc.), it should be made an invariable rule in carrying out the separation, first to follow through, to the point of identification, those colouring matters that seem to be present in largest proportion. The course to be pursued in dealing with the smaller fractions will then be more clearly indicated.

SEC. 1.—The solution containing the colouring matter is treated with enough strong sodium chloride solution to bring the salt concentration to about 5 or 6% and is then shaken out with 20 c.c. or more of amyl alcohol. If a considerable amount of colouring matter is taken up, the extraction is repeated once or twice, the different portions of solvent being finally combined. The amyl alcohol, if coloured, is washed once or twice with small portions of 5% salt solution, and these washings, if they appear to contain any dye, are added to the original extracted solution. Any suspended solid matter that may separate may be considered also to belong to the aqueous solution.[1]

The amyl alcohol, if colourless or freed from colour by the washing, is discarded. Basic dyes and most of the acid colours of low sulphur

[1] The more systematic procedure described on pages 499–500 may be used, if preferred, for this and other similarly described extractions.

content are absent. If the amyl alcohol is coloured, it is treated as directed in section 10.

SEC. 2.—The extracted salt solution is treated with about one-half its volume of concentrated hydrochloric acid and is again shaken out with amyl alcohol, exactly as described in section 1. Should colouring matter be extracted, the combined portions of the solvent are washed once with diluted acid (1:2, approximately 4N), then reserved for treatment as stated under section 6. If the alcohol is colourless, and remains so after treatment with an excess of ammonia solution, it is discarded; and most of the strongly sulphonated azo colours are known to be absent. (When Naphthol Green B is present, compare section 18.)

SEC. 3.—The extracted acid salt solution, which may appear nearly colourless, is treated with ammonia until slightly alkaline, then made slightly acid with acetic acid. If it is now colourless, the absence of the strongly sulphonated triphenylmethane green and blue dyes is shown, and it is discarded. If it is coloured, and if the shade indicates the possible presence of green or blue colours, it is shaken out with dichlorhydrin. This solvent is slightly soluble in water, but an amount should be used so that the lower layer after separation will not measure more than 20 c.c. If colouring matter of bluish tint has been extracted, the mixture is again shaken out once or twice, and the combined portions of solvent washed with a little salt solution. The dichlorhydrin solution is then examined according to section 5.

SEC. 4.—The original mixture after the preceding extractions may still contain Acid Magenta, Caramel, and many natural colours, especially the glucosides (anthocyans) constituting the common fruit colours. Acid Magenta may be recognised by its reactions with nitrous acid, dyeing properties, etc. It may be separated, if desired, by adding hydrochloric acid so that the acidity is above that of N/4 hydrochloric acid solution (allowance must be made for the ammonium acetate present) and then shaking out with aniline. The aniline solution is washed with N/4 hydrochloric acid in salt solution of from 5 to 6% strength; and the dye then removed with water, perhaps after addition of some carbon tetrachloride. Before testing this magenta solution the dissolved aniline must be carefully removed by making alkaline and extracting several times with carbon tetrachloride, benzene, or other convenient solvent. Commercial Acid

Magenta is a somewhat variable mixture of sulphonates and may be expected to yield considerable fractions of lower sulphonated derivatives of greater relative solubility in organic solvents.

The acid yellows (No. 8 and No. 9), although chiefly extracted by amyl alcohol from the acid solution (sec. 2), always yield a large fraction in this group. When the coloration of the extracted acid salt mixture is entirely due to such products it will be orange red, becoming yellow on neutralisation, and will also show the characteristic reactions of the acid yellows with nitrous acid, etc.

Sec. 5.—The dichlorhydrin solution is diluted with three or four times its volume of carbon tetrachloride and the colour removed with a few small portions of water. The combined washings should be shaken out once with carbon tetrachloride to get rid of dissolved dichlorhydrin. The aqueous solution may contain the higher sulphonated triphenylmethane colours, or perhaps sulphonated induline. These dyes, like Acid Magenta, are accompanied by large amounts of subsidiary products, and their solubilities cannot be established with any definiteness. For their further differentiation compare their properties as shown in the tables.

Sec. 6.—The amyl alcohol extract of the strongly acid salt solution, if coloured, is washed four or five times with N/4 hydrochloric acid, the washings being kept separate. No. 108 and No. 692 predominate in the first washings, while the acidity is still high owing to hydrochloric acid dissolved in the amyl alcohol. No. 106, No. 107, and No. 94 come out in large proportion when the acidity of the lower layer, after the shaking, is below 0.7N (usually about the third washing). Obviously a stronger acid than N/4 may be used at first, but it is usually better in practice to wash with this concentration and refractionate if necessary. The dyes that may be present in the acid amyl alcohol extract show a gradual transition in their distribution ratios relative to amyl alcohol (and other like solvents) and hydrochloric acid of varying concentration. Consequently the acid normalities to be chosen in working with an unknown mixture must be selected somewhat according to probabilities.

Comparison of the appearance of the different washings usually will show whether more than one colour is present which is extracted by N/4 hydrochloric acid in considerable proportion. The amyl alcohol is reserved for the treatment described in section 7 or 8. No. 108 may be separated from Nos. 106, 107, and 94 by fractiona-

tion between N/2 hydrochloric acid and amyl alcohol. Nos. 692 and 8 can be separated similarly from Nos. 106, 107 and 94 with N/8 sulphuric acid and a mixture of equal volumes of amyl alcohol and petroleum spirit; although, since the acid is somewhat difficult to remove afterwards, the procedure is better adapted for separating the last-named dyes in pure condition than Nos. 692 and 8. For Nos. 106, 107, and 94, the amyl alcohol and petroleum spirit solution is washed with a little water to take out the dye. This solution is treated with one-half its volume or more of concentrated hydrochloric acid and is re-extracted with amyl alcohol. This latter solution may now be washed with a few portions of hydrochloric acid of from 4N to 6N strength to remove sulphuric acid. The dye is finally removed with a little water, and the colour obtained in pure condition (for the cyanide reaction, for example) by evaporation to dryness on the steam bath. The dyes in the sulphuric acid solution are best separated by aniline but the final removal of this solvent is tedious. No. 94 must be separated from No. 106 and No. 107 by aniline and N/4 hydrochloric acid in 5 or 6% salt solution. After the fractionation the dissolved aniline in the solutions must be carefully removed by several extractions with carbon tetrachloride or other convenient solvent from the faintly alkaline solution.

Commercial No. 692 and No. 8 are made by direct sulphonation of colouring matters and are rather indefinite in composition. It will often be more convenient to divide the solutions of the colours of this group and to destroy different dyes in the various portions. By cautious treatment with "Blankite" ($Na_2S_2O_4$) in acid solution, subsequently shaking with air to restore the blue, No. 692 may be separated from the azo colours.

By reduction in ammoniacal solution, avoiding excess of "Blankite," No. 106 and No. 107 may be destroyed, whilst No. 8 is merely converted into the hydrazo compound and may be restored by shaking with air. No. 692 is destroyed by warming in acid solution containing a little urea and a drop of sodium nitrite solution, whilst Nos. 106, 107, and 108 are scarcely attacked. The cyanide reaction is best suited for the examination of mixtures of No. 106 and No. 107.

The dyes of this group, because of their ready solubility in water and fruit juices, are well adapted and largely employed for food colouring. Hence the application of the data given in the solubility table,

etc., has been indicated rather more fully here than for the other classes.

SEC. 7.—The amyl alcohol extract, after being washed with N/4 hydrochloric acid, may be similarly washed with N/16 hydrochloric acid; although, unless No. 14 or No. 188 appear to be present, this step usually will be omitted.

SEC. 8.—The amyl alcohol is now measured, diluted with an equal volume of low-boiling point petroleum spirit and washed first with N/4 hydrochloric acid two or three times, then similarly with N/16 hydrochloric acid, with N/64 hydrochloric acid, with N/64 acetic acid, and finally with N/64 sodium hydroxide. The dyes separated here include a large number of individuals, and the treatment most desirable for any given mixture can best be judged after reference to the tables, pages 464 to 476. Obviously, the normalities stated are chosen somewhat arbitrarily, any two dyes contiguous in the table usually differing little from each other in solubility. When the appearance etc., of the different fractions indicate the presence of more than one dye, the colouring matters must be obtained in pure condition by refractionation. Although the acid amyl alcohol extract, after dilution with gasoline, appears to yield all its colour to the acid washings, it must nevertheless be shaken with the alkaline solution before being discarded, since a number of the weakly acid colouring matters (most of which, it is true, do not properly come in this group) are nearly colourless when dissolved in the neutral or acid organic solvent.

Naphthol yellow S, which predominates in the first strongly acid washings, is also nearly colourless in acid solutions, and a portion from these solutions must always be tested for this dye by making it 2N with hydrochloric acid and shaking with washed ethyl acetate. If the separated solvent is found by treatment with alkali to have taken up a yellow dye, the remainder of the fractions containing it are treated in the same way with the acetate. Although the washings of low acidity may contain some colouring matters, the major portion of such dyes will be in the amyl alcohol extract of the neutral salt solution. It is best, therefore, to set aside the N/64 acetic acid and the N/64 sodium hydroxide washings until after the examination of the netraul salt and amyl alcohol extract has been made; or these solutions may be mixed with the corresponding ones obtained by the processes outlined in sections 11 and 12 and may be

worked up with them. Or, finally, the amyl alcohol and petroleum spirit mixture, after washing with N/64 hydrochloric acid, may be reserved and combined with the similar mixture described in section 10.

SEC. 9.—For the separation by chemical means of closely similar dyes of these groups some of the more useful general methods may be indicated here.

The reaction with cyanide (page 491) may be used for the separation of R-salt derivatives (Nos. 55, 56, 65, 15) from mixtures with isomers.

Methods based on reduction and subsequent oxidation are applicable for the destruction of azo and nitro colours in presence of most other classes of colours, as indicated in the tables of Weingärtner and of Rota.

By cautious reduction in sodium carbonate or ammoniacal solution oxyazo dyes tend to be attacked more rapidly than aminoazo dyes. It must be remembered, however, that new dyes may also be formed by partial reduction in the case of polyazo or nitroazo derivatives.

The halogenated fluorescein derivatives are much more resistant to bromine in acid solution than are most other colours. They tend, however, to add bromine unless fully substituted. Most of the azo dyes are much more readily destroyed by bromine in alkaline solution than is Naphthol yellow S. Mixtures are made N/4 or above with sodium carbonate and are treated with dilute bromine water very cautiously until the azo dye is just destroyed or until the solution has become a clear yellow. Hydrazine sulphate is now added quickly to destroy excess of bromine, the mixture is finally acidified, and the yellow purified by extraction with an immiscible solvent. This procedure is seldom so satisfactory as the regular extraction with ethyl acetate or amyl acetate, and is not applicable in the presence of Nos. 62, 64, 65, and 188, which form intensely blue substances by this treatment.

SEC. 10.—The amyl alcohol extract obtained by shaking out the original mixture after adding 5 or 6% salt will contain practically all of any basic dyes present. Most of the acid dyes of low sulphur, content are also almost completely extracted. The extract is measured, diluted with an equal volume of petroleum spirit, then washed a few times with N/64 hydrochloric acid. The washings, if coloured,

are treated as directed in section 11. The extract is next shaken out with N/64 acetic acid, these washings being treated according to section 12. Eosines and (in general) colouring matters which are unsulphonated phenolic compounds are now removed by a few portions of N/64 sodium hydroxide solution, this fraction being treated according to section 13. The amyl alcohol and petroleum spirit mixture is finally washed once with very dilute acetic acid, and, if still containing any significant amount of colouring matter, is evaporated to dryness on the steam bath, the residue being examined according to section 14.

SEC. 11.—The washings of N/64 hydrochloric acid (sec. 10) are tested for basic dyes by making a small portion alkaline with sodium hydroxide, shaking with ether, then treating the ether solution, which is usually colourless, with dilute acetic acid.[1] If the acid becomes coloured, indicating the presence of basic dyes, the alkaline test portion may be shaken out once or twice more to determine whether acid dyes are also present in this fraction. If these tests indicate the presence of both acid and basic colours, the acid colours must be removed by making the principal part of the N/64 hydrochloric acid extract alkaline (normal with sodium hydroxide) and extracting with ether. From the combined ether portions the basic dyes are removed by washing—first with N/64 acetic acid, finally with dilute hydrochloric acid. This treatment should be omitted if acid dyes are absent, since most basic colours are unstable in alkaline solutions, Auramine, especially, suffering decomposition rapidly. The basic colours may be further fractionated from amyl alcohol with dilute hydrochloric acid, from ether with very dilute alkali, etc.

The alkaline solution, after removal of the basic dyes with ether, is made about normal with hydrochloric acid and is shaken out with amyl alcohol and petroleum spirit mixture. Any colouring matter extracted here probably will be a minor portion of a dye already obtained by the procedure described under section 8, and its further fractionation will be carried out as stated in that paragraph; or the solution containing it may be combined with the similar solution obtained by the procedure outlined in section 8 and both fractionated together.

[1] O. N. Witt, *Zeitsch. anal. Chem.*, 1887, **26**, 100. Weingärtner, *Zeitsch. anal. Chem.*, 1888, **27**, 232.

The hydrochloric acid solution is again partly neutralised by addition of sodium hydroxide (to fourth-normal or less) and is shaken out with a mixture of 3 volumes carbon tetrachloride and 1 volume dichlorhydrin to extract the lower sulphonated triphenylmethane dyes. These may be obtained in aqueous solution again, by washing out with water after adding more carbon tetrachloride.

SEC. 12.—The acetic acid solution obtained by the procedure described in section 10 will contain the chief part of any monosulphonated monazo dyes present. Such colours may be further fractionated with amyl alcohol and sodium carbonate, with ether and dilute hydrochloric acid, etc.

The colouring matters of this group may appear in small proportion in the fractionation described in section 8, and obviously the similar solutions there described may be combined with the acetic acid solution obtained as described in section 10.

SEC. 13.—The main part of the eosine dyes, and of unsulphonated water-soluble acid colours in general, will be found in the N/64 sodium hydroxide solution obtained by the extraction described in section 10. A large proportion of the natural colouring substances also appear here.

The eosine dyes may be fractionated between N/1 sodium hydroxide amyl alcohol or amyl alcohol petroleum spirit mixture (3:1).

The acid dyes also having basic tendencies, as Fluoresceine (No. 510), Metanil Yellow (No. 95), differ from the others in being extracted from strongly acid solutions in smaller amounts than from weakly acid solutions, and this property offers a suitable means for their separation (page 469). These colours, as already pointed out, may be obtained, though in most cases in very small proportion, in the amyl alcohol petroleum spirit solution obtained by the extraction described in section 8.

SEC. 14.—The residue mentioned in section 10 is moistened with a drop of alcohol, and then some ether and N/64 hydrochloric acid are needed. The mixture is poured into a separatory funnel and is shaken. The aqueous layer is drawn off, and if dyes colouring the aqueous solution were present, the ether is washed a few times further with the N/64 acid, to remove them. The acid solution contains the rhodamines, perhaps also some of the basic azo colors.

The ether solution, if coloured, is now washed a few times with 4N hydrochloric acid, the washings being neutralised at once and reserved for treatment according to section 15.

The ether is finally washed a few times with water to remove acid; then it is taken to dryness on the steam bath and the residue treated according to section 16.

SEC. 15.—This group, consisting of oil-soluble colours, may be further separated by taking up the dye in gasoline or petroleum spirit from the neutralised solution obtained as described in section 14, and fractionating from this solvent with methyl alcohol (70% or above).

SEC. 16.—The residue containing Sudans, etc., may be treated with measured quantities of methyl alcohol, water, and sodium hydroxide in the proportions necessary to bring the mixture to N/4 alkalinity in 80% alcohol; the solution then may be shaken out twice with petroleum spirit. Quinoline yellow and α-naphthol derivatives remain chiefly in the alkaline solvent. The petroleum spirit portions are combined, then treated as described in section 17.

SEC. 17.—The dyes of this group may be separated further by washing the petroleum spirit solution with sulphuric acid of different concentrations. Yellow A. B. and Yellow O. B. are quite sensitive to the traces of aldehydes, etc., present in most solvents, so that the original mixtures containing these dyes should be treated with purified petroleum spirit, and the solutions thus obtained washed or fractionated with dilute sulphuric acid as indicated on pages 448 and 476. An approximate separation of the two dyes can be made by fractionating between 13N sulphuric acid and petroleum spirit. (*J. Ind. Eng. Chem.*, 1920, **12**, 883).

SEC. 18.—When Naphthol Green B is present the salt solution should not be made strongly acid, since small amounts of this dye decompose quickly in acid solution. When its presence is suspected, the neutral salt mixture may be first extracted with dichlorhydrin, washed once with benzene to remove the dissolved solvent, made N/2 with hydrochloric acid, and then shaken out with aniline. (It is best to add the solvent before the acid.) From the aniline solution the dyes may be fractionated by shaking out with 5 or 6% salt solution which contains hydrochloric acid varying from N/4 to N/64.

No outline in the form of a key can be so useful as a table of solubilities. The solvents and the order in which they are to be used obviously may be varied when the analyst has information regarding the source and appearance of a sample. For example, in the case of the red colour solution obtained from commercial cocktail cherries known to be ordinarily coloured with Erythrosine, it would be better

to make such solution acid and shake it out with ether first, the complete extraction of the dye indicating at once the absence of all excepting one group of colours. The table on pages 464–476 names about 130 coal tar dyes, including almost all that have been widely used as food colours or that have been mentioned in published reports as suitable for such purposes. The corresponding solubilities of the large number of isomeric and closely related colouring matters may be predicted with moderate accuracy, so that the data may be applied for the separation or differentiation of most of the simpler colouring matters now on the market. (See *U. S. Tariff Commission Bulletin* 31, *Tariff Information Series.*)

The writer prefers, after fractionating the colours into the main groups as just described, to try the bromine test, page 488. The behaviour with acids has already been seen in the course of the separation, and that with alkali can be quickly ascertained. Ordinarily these will indicate the fraction to contain but one colouring matter. This is then dyed out from a portion of the solution, and its shade and reaction on the fibre with reagents are compared with standards or with statements in the tables, in which, to facilitate comparison, the dyes have been arranged in the order of their solubility. Since the colour changes produced in dilute dye solutions, by addition of acids and alkalies, are closely parallel to those shown by the same reagents on the dyed wool, a single table indicating the reactions on the fiber is sufficient in practice.

Even when the tests have indicated that the fraction still contains a mixture of dyes, they will have shown, in most cases, the absence of many colours of the group, and will have indicated positively which colours are probably present.

If coal tar dyes have been found, the treatment for their separation will have given much information as to natural colours that may be present. Many of the natural colours will have been separated in fairly pure condition by the fractionation, and the solutions obtained will be ready for identification tests. Obviously, no essential difference exists between these and the so-called coal-tar colours; as a class, however, they show much less tendency to dye wool than do the common synthetic colouring matters, and in addition are in many cases so sensitive to alkalies as to be completely destroyed in the double-dyeing test; *i. e.*, by dyeing, stripping the fibre with dilute ammonia, acidifying, and dyeing again. The preliminary dyeing with wool described on page 431 serves fairly well in practice as a first

indication of the course to be followed; but when for some reason the results obtained are not decisive, the treatment for coal-tar colours with immiscible solvents should be carried out with consideration of solubilities of the colouring matters described in the foot-notes in the tables of solubilities. The crude products constituting the commercial natural colouring matters in most cases are mixtures containing several closely related chemical individuals. These may have different solubilities, but usually they contain the same chromophore groups, and are of closely similar shade. In practice, the analyst will scarcely attempt a full separation, but having identified the colouring matter in one fraction, can judge as to the likelihood of the other substances present being derived from the same source. The natural colours as a class do not contain strongly acidic groups, and their distribution ratios between immiscible solvents do not show wide variations with the acidity of the latter, at least not within convenient limits. The colouring matters of Cochineal and Turmeric give less trouble than the others, partly because they are less heterogeneous.

When coal-tar dyes are absent, and it is desired to fractionate with immiscible solvents, it is best to begin extraction with neutral solutions; perhaps first using ether (petroleum spirit is better when chlorophyll and the accompanying leaf colours are to be separated). The final extraction may be made with amyl alcohol from acid solution, but it is of no advantage to have the acidity high, not, perhaps, above N/32. The anthocyans which constitute the colouring matters of the common red fruits and flowers are glucosides, and are extracted from acid solution only in very small amount by amyl alcohol and similar solvents. Their neutral solutions may be treated with excess of lead acetate solution (normal salt) when practically all of the glucoside will be precipitated. The mixture may be centrifuged and the precipitate washed in the centrifuge tubes with several portions of water until sugar and similar soluble substances have been removed. The precipitate may then be dissolved in hydrochloric acid of 10 or 15% strength. After whirling again in the centrifuge to separate the lead chloride thrown out of the solution, the clear red liquid is shaken out once or twice with amyl alcohol to remove various extractives soluble in this substance. It may then be boiled for a short time, by which means the glucoside is hydrolysed, the derived colouring matter or anthocyanidin, being produced. This may now be extracted and obtained in fairly pure

solution by shaking out with amyl alcohol. The anthocyanidins, according to Willstaetter, (*Sitzb. kgl. Preuss.*, 1914, **12**, 402–411. *Ber.*, 1918, 784) are oxonium bases, containing also acidic phenolic groups. They are not very readily soluble in amyl alcohol, though relatively more so than in aqueous liquids. For further papers by Willstaetter and co-workers see *Annalen* 1915, **408**, 1–158; 1919, **412**, 164, 195; *Ber. Deut. Bot. Ges.*, 1915, **25**, 447.

The colouring principles of saffron and of Persian berries also consist chiefly of glucosides, though the lead salts of these are relatively more soluble. These glucosides also are readily hydrolysed by boiling with acid, but the change in case of saffron is attended with destruction of much colouring matter, at least when the hydrolysis is carried out in the ordinary manner, with free access of air. Berberine is said to be the only common natural basic colouring matter and it is seldom, if ever, found in food products.

The yellow and green colouring matters present in green leaves and similar plant structures, are extracted from neutral aqueous mixtures by ether, and are not removed from this solvent by washing with dilute alkali. Identical or closely related colouring matters are also found in egg yolk, (Willstaetter and Escher, *Zeit. physiol. Chem.*, 1912, **76**, 214) fats and oils, (See Palmer and Eckles *Mo. Sta. Research Bull.* Nos. 9, 10, 11, 12), carrots and tomatoes (Willstaetter and Stoll, *Untersuchungen ueber Chlorophyll*, Berlin, 1913).

Colouring matters of alkanet, annatto, turmeric, and of the red dye-woods (sandalwood, camwood, and barwood) are very readily and completely extracted by ether from slightly acid solutions. The flavonol colouring matters of quercitron and Persian berries (after hydrolysis), and of fustic, as also the colouring matter of Brazil wood and the green derivatives formed from chlorophyll by alkali treatment, are taken up in very large proportion by ether from slightly acid solutions.

The colouring matters of logwood, of archil, of saffron, and of cochineal are extracted in relatively small amount by ether from slightly acid solutions, but are largely taken up by amyl alcohol.

Caramel and the anthocyans constituting the red colouring matters of most common fruits are extracted in relatively small proportion by amyl alcohol from acid solutions. Ammoniacal cochineal (carmine) is similar, but the ordinary colouring matter is readily re-formed on standing with hydrochloric acid.

NUMBERS BY WHICH DYES ARE DESIGNATED IN DIFFERENT PUBLISHED TABLES

[Under "G." numbers refer to *A Systematic Survey of the Organic Colouring Matters*, by A. G. Green, founded on the German of Drs. G. Schultz and P. Julius, London and New York, 1904; under "S." to *Farbstofftabellen*, by Dr. Gustav Schultz, Berlin, 1911–1914, under "M." to Mulliken's *A Method for the Identification of Pure Organic Compounds*, vol. 3, New York, 1910. One of the common names is also given.] Serial numbers are given in the first column to facilitate reference to the tables of properties.

No.	Colour	G.	S.	M.
1	Acid Magenta	462	524	245
2	Light Green S F Bluish	434	504	.....
3	Light Green S F Yellowish	435	505	257
4	Erioglaucine A	436	506	287
5	Cyanol Extra	439	546	285
6	Wool Green S	491	566	279
7	Patent Blue	440	543	277
8	Nigrosine Soluble	602	700	122
9	Ponceau 6 R	108	170	846
10	Acid Yellow G	8	137	918
11	Fast Yellow R	9	149	.....
12	Brilliant Yellow S	89	142	.....
13	Indigo Carmine	692	877	94
14	Sun Yellow	399	9	166
15	New Coccin	106	169	826
16	Amaranth	107	168	811
17	Tartrazine	94	23	948
18	Naphthol Green B	398	4	951
19	Azocarmine B	605	673	75
20	Azocarmine G	604	672	71
21	Naphthol Black B	188	272	984
22	Orange G	14	38	.....
23	Fast Acid Fuchsin B	21	41	.....
24	Chicago Blue 6 B	318	424	575
25	Chromotrope 2 R	20	40	.....
26	Azofuchsine G	93	146	757
27	Soluble Blue	480	539	212
28	Palatine Scarlet	53	81	.....
29	Ponceau 2 R	55	82	834
30	Fast Red E	105	166	.....
31	Naphthol Yellow S	4	7	946
32	Cochineal	706	932	774
33	Ponceau 3 R	56	83	833
34	Palatine Red	62	109	.....
35	Crystal Ponceau	64	113	843
36	Bordeaux B	65	112	778
37	Azorubine	103	163	783
38	Fast Brown	130	213	.....
39	Crocein Scarlet O extra	164	251	...
40	Quinoline Yellow water-soluble	667	613	.....
41	Crocein Scarlet 8 B	169	255	802
42	Biebrich Scarlet	163	247	800
43	Bordeaux G	170	254	1133
44	Resorcine Yellow	84	143	830
45	Brilliant Crocein M	146	227	...
46	Azo Blue	287	377	689
47	Erika B	78	121	.....
48	Azolitmin	710	934	.....
49	Alizarine Red S	546	780	1111
50	Picric Acid	1	5	947
51	Violamine R	507	582	1101
52	Brilliant Yellow	328	303	.....
53	Rosinduline 2 G	606	674	78
54	Cloth Red B	154	236	371
55	Orange I	85	144	871
56	Croceine Orange	13	37	877
57	Orange 2	86	145	872
58	Orange R	97	151	.....
59	Scarlet G R	54	...	.....
60	Chrysophenin	329	304	524
61	Resorcine Brown	137	211	451
62	Bordeaux B X	157	237	1132
63	Metanil Yellow	95	134	901

No.	Colour	G.	S.	M.
64	Orange IV	88	139	900
65	Azoflavine	92	140	910
66	Fast Brown N	101	160	849
67	Fast Red A	102	161	777
68	Rosolic Acid	483	555	249
69	Uranin	510	585	1141
70	Metachrome Orange R	26	58	893
71	Chrysamine G	220	342	1061
72	Chrysamine R	269	394	1060
73	Eosine	512	587	1129
74	Saffrosin	515	590	1114
75	Erythrosine G	516	591	.
76	Erythrosine B	517	592	1113
77	Phloxine	518	593	1107
78	Rose Bengale	520	595	1109
79	Eosin 10 B	521	596	1112
80	Rose Bengale 3 B	523	597	1103
81	Victoria Yellow	2	...	...
82	Martius Yellow	3	6	945
83	Aurantia	6	...	897
84	Alizarine	534	778	905
85	Curcumin	707	927	535
86	Sudan G	10	35	536
87	Formyl Violet S 4 B	468	530	307
88	Acid Violet N	464	527	309
89	Night Green 2 B	438	503	265
90	Guinea Green B	433	502	259
91	Patent Blue A	442	545	278
92	Methyl Alkali Blue	476	535	196
93	Congo Red	240	307	412
94	Benzopurpurin 4 B	277	363	1020
95	Alizarine Blue	562	803	1168
96	Thioflavin T	658	618	1084
97	Rhodamine S	496	570	143
98	Methylene Blue	650	659	23
99	New Blue	639	649	34
100	Safranine	584	679	8
101	Fuchsine	448	512	139
102	Auramine O	425	493	1085
103	Auramine G	426	494	.
104	Methyl Violet	451	515	232
105	Crystal Violet	452	516	229
106	Malachite Green	427	495	177
107	Malachite Green G	428	499	170
108	Bismarck Brown	197	283	454
109	Bismarck Brown R	201	284	1030
110	Chrysoidine	17	33	500
111	Chrysoidine R	18	34	.
112	Rhodamine 3 B	505	574	1002
113	Irisamine G	499	576	1012
114	Rhodamine B	504	573	1001
115	Rhodamine G	502	572	1101
116	Butter Yellow	16	32	
117	Anilin Yellow	7	31	932
118	Yellow Fat Colour	..	68	.
121	Quinoline Yellow spirit-soluble	666	612	
....				
123	Sudan Brown	59	105	...
125	Sudan I	11	36	887
126	Sudan II	49	76	1026
127	Carminaph Garnet	60	106	.
128	Sudan III	143	223	..
129	Sudan IV	..	232	839
130	Para Red	31	56	.....
....	.....	...	...	.....

TABLE 1.—EXTRACTION OF COLOURING SUBSTANCES FROM AQUEOUS SOLUTIONS BY IMMISCIBLE SOLVENTS

Statements refer in all cases to the amount of dye taken up by the organic solvent when this is shaken with an equal volume of the 0.01 % solution of the colouring matter in the various aqueous liquids.

The colouring matters are designated by numbers as given in the tables in "A Systematic Survey of the Organic Colouring Matters." By A. G. Green. Founded on the German of Drs. G. Schultz and P. Julius. Second edition, London and New York, 1904.

For names and references to other large published tables, see page 463.

Numbers denoting permitted dyes are in bold-faced type; natural colours included in the body of the tables are in italics. The numbers of the dyes more commonly found in foods are starred, three stars indicating those dyes most frequently used.

Serial numbers are given in the first column to facilitate reference to the tables of properties, pages 478–481 and 483–491.

Number	Colouring matter	Colour of water solution	Amyl alcohol and approximately normal (5–6 %) sodium chloride solution	Amyl alcohol and hydrochloric acid solutions of varying normality	Amyl alcohol gasoline mixture (1:1) and dilute hydrochloric acid	Ether	Dichlorhydrin and N sodium chloride solution	Other data
1	*462	Crimson; with HCl, little change.	Little or none extracted.	Little or none extracted from acid of concentration 4N or less.	Little or none extracted from acid of normal concentration or more dilute.	Little or none extracted from acid, neutral, or alkaline solutions.	Little extracted.	
2	434	Green; strongly acid solution, brownish yellow to colourless according to dye concentration.					Larger part or almost all extracted from 5–20 % salt solution.	With a mixture of 1 volume washed dichlorhydrin, 3 volumes carbon tetrachloride, and with N/64 acetic acid: **435**, 439, very little extracted; 491, little extracted; 440, one-half or more extracted.
3	**435**							
4	*436	Greenish blue; with HCl, same reaction as **435**.						
5	439	Blue; with HCl, same reaction as **435**.						
6	491	Greenish blue; with HCl, same reaction as **435**.						
7	440	Blue; with HCl, same reaction as **435**.	Less than one-half extracted.					
8	**602	Blackish blue; with HCl, little change.	Little extracted.					
9	**108	Magenta red; with HCl, little change.	Little or none extracted.	4N, larger part extracted; N, little extracted.			Little or none extracted.	
10	***8	Yellow; with HCl, red.		4N, larger part extracted; N, one-half or				With 8 N H_2SO_4 and amyl alcohol gasoline mixture (equal volumes): 8, little
11	9	Yellow; with HCl, red.						
12	**89	Yellow; with HCl, red.						

13	**692**	Blue; with HCl, little change.		less extracted; N/4 and below, little or none extracted.				extracted; **692**, larger part not extracted; 89, more than one-half extracted; **107**, 106, and **94**, larger part extracted. With anilin and acid 5 per cent. sodium chloride solution (normality stated is that of the HCl before shaking): N/2, **692**, **107**, and **94** almost all extracted; N/4, **94**, less than one-half extracted; 8, 89, **692**, **107**, 106, and 398, almost all extracted; N/16, **94**, little extracted; 89, less than one-half extracted; **602**, about one-half extracted; 106, **107**, more than one-half extracted; 398, chief part extracted; N/64, **107**, 106, **94**, and 398, little or none extracted.
14	399	Yellow; with HCl, little change.						
15	***106	Scarlet red; with HCl, little change.		4N, almost all extracted; N, larger part extracted; N/4 and below, little or none extracted.				
16	**107**	Magenta red; with HCl, little change.						

Most of the dyes described on this page are usually accompanied by considerable amounts of subsidiary products, especially of substances similar chemically but of a different degree of sulphonation.

Caramel and the glucosides constituting the colouring matters of most red fruits resemble Acid Magenta (No. 462) in being extracted in small proportion only, by amyl alcohol or dichlorhydrin from the various aqueous solutions.

TABLE 1.—EXTRACTION OF COLOURING SUBSTANCES FROM AQUEOUS SOLUTIONS BY IMMISCIBLE SOLVENTS.—(*Continued*)

Number	Colouring matter	Colour of water solution	Amyl alcohol and approximately normal (5–6 %) sodium chloride solution	Amyl alcohol and hydrochloric acid of varying concentration	Amyl alcohol gasoline mixture (1:1) and hydrochloric acid of varying normality	Ether	Dichlorhydrin carbon tetrachloride mixtures (equal volumes; also 1 volume dichlor. +3 volumes CCl_4)	Washed ethyl acetate and 2N hydrochloric acid
17	94	Yellow; with HCl, no change.	Little or none extracted.	As stated for 106 and **107**, p. 465.		Little or none extracted by ether from neutral acid or alkaline solutions.	Little or none extracted by mixture 1:1 or by mixture 1:3, from N/4 HCl or N/64 acetic acid.	Very little or none extracted by ethyl acetate.
18	*398	Green; with HCl, gradually yellow.		Similar to dyes just preceding, but rapidly decomposed.				
19	605	Red; with HCl, little change.		½N and N; larger part extracted; N/4 less than one-half extracted.				
20	604	Red; with HCl, little change.						
21	188	Violet; with HCl, bluer.		N and above, almost all extracted; N/4, about one-half extracted; N/16 and below, little extracted.	4N, larger part extracted; N and below, little or none extracted.			
22	***14	Orange; with HCl, no change.						
23	21	Magenta red; with HCl, slightly yellower.		4N and N, chief part extracted; N/4, more than one-half; N/16, less than one-half.	4N, larger part not extracted; N, little extracted.			
24	318	Blue; with HCl, little change.		N and above, almost all extracted; N/4, larger part extracted; N/64, less than one-half extracted.	Behaves as stated for 14 and 188.			
25	20	Magenta red; with HCl, no change.			4N, almost all extracted; N, larger part not extracted; N/16 and below, little or none extracted.			
26	93	Bluish red; with HCl yellower.						
27	***480	Blue; with HCl, slightly paler.			4N, less than one-half extracted; N and below, little extracted.			

28	*53	Scarlet; with HCl, little change.		N/4 and above, almost all extracted; N/64, one-half or more extracted.	N, one-half or less extracted; N/16 and below, little or none extracted.			
29	*55	Scarlet; with HCl, little change.						
30	105	Scarlet; with HCl, little change.						
31	4	Yellow; with HCl, almost colourless.		The same as above, but large part extracted at N/64.			As stated for 56, 62, etc., p. 468.	Larger part extracted.
32	***706	Orange red; with HCl, little change.						Little extracted.

TABLE 1.—EXTRACTION OF COLOURING SUBSTANCES FROM AQUEOUS SOLUTIONS BY IMMISCIBLE SOLVENTS.—(*Continued*)

No.	Colouring matter	Colour of water solution	Amyl alcohol and approximately normal (5–6 %) sodium chloride solution	Amyl alcohol and hydrochloric acid of varying concentration	Amyl alcohol gasoline mixture (1:1) and hydrochloric acid of varying concentration	Ether	Dichlorhydrin-carbon tetrachloride mixtures	Washed ethyl acetate and 2 N hydrochloric acid	Amyl alcohol gasoline mixture (1:1) and dilute sodium hydroxide solution
33	56	Cherry red; with HCl, no change.	Little or none extracted.	N/16 and above, almost all extracted; N/64, larger part extracted.	N, more than one-half extracted; N/16 and below, little or none extracted.	Little or none extracted from acid, neutral, or alkaline solutions.	Little or none extracted by mixture 3 volumes CCl_4 and 1 volume washed dichlorhydrin, from N/64 acetic acid.	Little extracted.	Little or none extracted from N/64 NaOH solutions.
34	*62	Bluish red; with HCl, little change.							
35	**64	Scarlet, with HCl; slightly darker.							
36	*65	Magenta red; with HCl, little change.							
37	**103	Same reaction as 65.							
38	139	Brown; with HCl, little change.							
39	164	Orange red; with HCl, darker; finally violet.							
40	**667	Yellow; with HCl, no change.							
41	*169	Scarlet; with HCl, darker; finally violet.	Intermediate in behaviour between preceding and succeeding groups. Of most of the dyes, more than one-half is extracted.	Almost all extracted at N/64 and above.	N, larger part extracted; N/16, larger part not extracted; N/64, little extracted.				
42	163	Orange red; with HCl, darker.							
43	170	Red; with HCl, darker; finally blue-violet.							
44	84	Orange yellow; with HCl, little change.							
45	146	Cherry red; with HCl, darker; finally blue-violet.							
46	287	Violet; with HCl, little change.							
47	78	Red.							
48	***710	Violet.							

The colouring matters described on the lower half of this page are soluble with difficulty in both layers with most acid mixtures. This is especially true of Nos. 78, 710, and 287, which may be precipitated to some extent.

SOLVENTS.—(*Continued*)

No.	Colouring matter	Colour of water solution	Amyl alcohol and normal (5–6 %) sodium chloride solution	Amyl alcohol and dilute hydrochloric acid	Amyl alcohol gasoline mixture (1:1) and dilute hydrochloric acid	Amyl alcohol gasoline (1:1) and N/64 acetic acid	Ether and hydrochloric acid of varying concentration	Dichlorhydrin-carbon tetrachloride mixture (1:3) and N/64 acetic acid	Amyl alcohol and N sodium carbonate solution	Amyl alcohol gasoline mixture (1:1) and N/64 sodium hydroxide	Ether and dilute sodium hydroxide solution
49	*546	Orange yellow.	Intermediate in solubility between preceding and succeeding groups.	Almost all extracted at normalities N/64 and above.	As stated for 169, 163, etc., p. 468.			Little extracted.		Little or none extracted.	Little or none extracted by ether from solutions N to N/64.
50	1	Yellow.									
51	507	Violet red.			N more than one-half extracted; N/16, one-half or less extracted; N/64, larger part not extracted.						
52	328	Yellow.									
53	606	Scarlet.	Chief part extracted.								
54	154	Magenta red.			N/16, one-half or more extracted; N/64 less than one-half extracted.						
55	85	Orange.				Little extracted.			Larger part not extracted.		
56	**13	Orange.	Almost all extracted.		N/16 and above, almost all extracted; N/64, larger part extracted.			Chief part not extracted.	Almost all extracted.		
57	***86	Orange.									
58	97	Orange.									
59	54	Reddish orange.									
60	329	Orange yellow.									
61	137	Brown.				Less than one-half extracted; except, 157, more than one-half extracted.		One-half or more extracted.			
62	157	Brownish red.									
63	*95	Orange yellow.					4 N, very little extracted; N, less than one-half extracted; N/64, little extracted.			Less than one-half extracted.	
64	88	Orange yellow.									

TABLE 1.—EXTRACTION OF COLOURING SUBSTANCES FROM AQUEOUS SOLUTIONS BY IMMISCIBLE SOLVENTS.—(*Continued*)

No.	Colouring matter	Colour of water solution	Amyl alcohol and approximately normal (5–6 %) sodium chloride solution	Amyl alcohol and dilute hydrochloric acid	Amyl alcohol gasoline mixture (1:1) and dilute hydrochloric acid	Amyl alcohol gasoline (1:1) and N/64 acetic acid	Ether and hydrochloric acid of varying concentration	Amyl alcohol gasoline mixture (1:1) and N/64 sodium hydroxide solution	Ether and sodium hydroxide solution	Amyl alcohol and N sodium hydroxide solution	Amyl alcohol gasoline (3:1) and N sodium hydroxide solution
65	*92	Yellow.	Almost all extracted.	All or nearly all extracted at N/64 and above.	Almost all extracted at N/64 and above.	Less than one-half extracted.	4 N and N, larger part extracted; N/64, little extracted.	Larger part not extracted.	Little or none extracted by ether, N to N/64.		
66	101	Brown.						Little extracted.			
67	102	Red.									
68	483	Red.	Less than one-half extracted.		Almost all extracted at N/64.	Almost all extracted.	4 N, very little extracted; N, little extracted; N/64 almost all extracted.				
69	**510	Yellow.									
70	26	Orange Yellow	Almost all extracted.		Almost all extracted at N/64 and above.		Almost all extracted at normalities N/64 and above.			Little extracted.	Very little extracted
71	220	Brownish yellow.								Precipitated.	Precipitated.
72	269	Brownish yellow.	One-half or more extracted by amyl alcohol.							Less than one-half extracted.	Very little extracted.
73	512	Red.									
74	515	Red.									
75	516	Red.									
76	517	Red.								Larger part extracted.	Less than one-half extracted.
77	518										
78	520	Bluish red.								Almost all extracted.	One-half or more extracted.
79	521	Bluish red.									
80	*523	Bluish red.	Almost all extracted								Almost all extracted.

No.	Colouring matter	Colour of water solution	Amyl alcohol and approximately normal (5–6 %) sodium chloride solution	Amyl alcohol and dilute hydrochloric acid	Amyl alcohol gasoline mixture (1:1) and dilute hydrochloric acid	Amyl alcohol gasoline mixture and N/64 acetic acid (mixture, 1:1)	Ether and dilute hydrochloric acid	Amyl alcohol gasoline mixture (1:1) and N/64 sodium hydroxide solution	Ether and dilute sodium hydroxide solution	Amyl Alcohol and normal sodium hydroxide solution	Amyl alcohol gasoline (3:1) and normal sodium hydroxide solution
81	2	Orange yellow.	Almost all extracted.	Almost all extracted at normalities $\frac{1}{64}$ and above.	Almost all extracted at normalities $\frac{1}{64}$ and above.	Almost all extracted.	Almost all extracted at normalities $\frac{1}{64}$ and above.	Little extracted (6, larger part not extracted).	Little extracted, N to N/64, except 6; N, almost all extracted; N/64, more than one-half extracted; 3, less than one-half extracted from N.		
82	*3	Yellow.									
83	6	Orange yellow.									
84	534	Alkaline solution, violet.									
85	***707	Alkaline solution, reddish brown.									
86	*10	Alkaline solution, orange.									

Colouring matters of fustic, of quercitron and Persian berries after hydrolysis, and of alkanet, have similar properties. They are extracted in large proportion by amyl alcohol and by amyl alcohol-gasoline mixture (equal volumes) from N/64 acetic acid or from hydrochloric acid, N/64 to N; also by ether from N/64 hydrochloric acid. They are not extracted in large proportion by amyl alcohol-gasoline mixture or by ether from N/64 sodium hydroxide solutions. Annatto, not alkali treated, behaves similarly, but is extracted in large proportion from N/64 sodium hydroxide by amyl alcohol gasoline mixture (1:1).

Colouring matters of barwood, camwood, and sandalwood resemble Nos. 483 and 510 somewhat in behaviour, but are less soluble in aqueous solvents. They are extracted almost completely by amyl alcohol from salt solution, and by amyl alcohol, amyl alcohol gasoline mixture (1:1), and ether from N/64 hydrochloric acid. They are not extracted by amyl alcohol gasoline mixture or ether from N/64 sodium hydroxide. From 4N hydrochloric acid the chief part of the colouring matter is not extracted by ether; from N hydrochloric acid the chief part is extracted. The colouring matter of Brazil wood is similar but relatively somewhat more soluble in aqueous solvents. That of logwood is also similar, but still more soluble in the water solutions. It is almost all extracted by amyl alcohol from salt solution or N/64 hydrochloric acid. The larger part is extracted by amyl alcohol gasoline mixture (equal volumes) from N/64 hydrochloric acid. The chief part is not extracted by ether from N/64 hydrochloric acid and very little from 4N hydrochloric acid. It is extracted in very small proportion from alkaline solution (N/64 sodium hydroxide) by ether or amyl alcohol gasoline mixture.

TABLE 1.—EXTRACTION OF COLOURING SUBSTANCES FROM AQUEOUS SOLUTIONS BY IMMISCIBLE SOLVENTS.—(*Continued*)

No.	Colouring matter	Colour of water solution	Amyl alcohol and approximately normal (5–6 %) sodium chloride solution	Amyl alcohol and dilute hydrochloric acid	Amyl alcohol gasoline mixture (1:1) and dilute hydrochloric acid (N/64)	Amyl alcohol gasoline (1:1) and N/64 acetic acid	Amyl alcohol gasoline (1:1) and N/64 sodium hydroxide	Ether and dilute acid (N/64 hydrochloric acid or acetic acid)	Ether and N/64 sodium hydroxide	Dichlorhydrin carbon tetrachloride mixtures and N/64 acetic acid
87	*468	Violet.	Nearly all extracted.	4N, very little extracted.	Little extracted.	Little extracted.	Little extracted.	Little or none extracted.	Little or none extracted.	Almost all extracted by mixture of equal volumes of solvents. By mixture 3 volumes CCl_4, 1 volume $C_3H_6OCl_2$, one-half or more extracted.
88	464	Violet.		N/64, almost all extracted.	More than one-half extracted.	More than one-half extracted.	Chief part extracted.			
89	438	Green.		N/64, almost all extracted.	Larger part not extracted.	Larger part not extracted.	Larger part not extracted.			
90	433	Green.		Partially extracted.						
91	442	Blue.		Almost all extracted at N/64.						
92	476	Blue.			Some extracted; remainder precipitated.			Little or none extracted; precipitated by HCl.		
93	240	Red.	Extracted from neutral or slightly alkaline solution.	Dye precipitated.	Dye precipitated.	Precipitated.	Not extracted.			
94	277	Brownish red.	Most of dye precipitated.	Most of dye precipitated; rest extracted.						
95	562	Alkaline solution blue to green.								
96	658	Yellow.	Almost all extracted.		Very little extracted.	Very little extracted.		Little or none extracted.		
97	496	Red.								
98	650	Blue.								
99	639	Violet.								
100	*584	Red.								
101	**448	Crimson.								

No.	Col-ouring matter	Colour of water solution	Amyl alcohol and normal sodium chloride solution	Amyl alcohol and hydro-chloric acid of varying concentration	Amyl alcohol gasoline mixture (1:1) and N/64 hydrochloric acid	Amyl alcohol gasoline mixture (1:1) and N/64 acetic acid	Ether and dilute hydro-chloric acid N/64 and above	Ether and dilute acetic acid	Ether and dilute sodium hydroxide solution
102 103	***425 426	Yellow. Yellow.	Almost all extracted.		Chief part not extracted.	Little extracted.	Little or none extracted.		Almost all extracted at N/64, but easily decomposed.
104 105	***451 452	Violet. Violet.			Less than one-half extracted.	Chief part not extracted.			N, chief part extracted.
106 107	**427 428	Green. Green.			Chief part not extracted.				
108 109	*197 *201	Brown. Brown.			Very little extracted.	Less than one-half extracted.		N, little extracted; N/64, as below.	Almost all extracted, N to N/64.
110 111	17 18	Orange. Orange.			Chief part not extracted.	More than one-half extracted.		N, larger part, not extracted; N/64, chief part extracted.	
112 113	505 499	Bluish red. Bluish red.			More than one-half extracted.				Almost all extracted at N.
114 115	***504 502	Bluish red. Bluish red.			Chief part extracted.	Almost all extracted.			Almost all extracted, N to N/64.

TABLE 1.—EXTRACTION OF COLOURING SUBSTANCES FROM AQUEOUS SOLUTIONS BY IMMISCIBLE SOLVENTS.—(*Continued*)

No.		Colouring matter	Colour of ether solution	Amyl alcohol and various aqueous solvents	Amyl alcohol gasoline mixture (1:1)	Ether and dilute hydrochloric acid (colours are completely extracted by ether from dilute acetic acid, N to N/64)	Gasoline and methyl alcohol of varying concentration (concentrations are by volume)			
							90 %	80 %	70 %	80 % N/4 with sodium hydroxide
116	**16	Dimethylamino-azo-benzene.	Orange yellow.	Oil-soluble colours insoluble in water extracted by amyl alcohol from aqueous solvents almost completely.	Extracted from water, N/64 acetic, or N/64 HCl.	4N, very little extracted; N/64, almost all extracted.	Larger part not extracted.	Less than one-half extracted.	Almost all extracted.	About one-half extracted.
117	7	Amino-azo-benzene.	Orange yellow.				Very little extracted.	Very little extracted.	Very little extracted.	Very little extracted.
118	*	Amino-azo-*o*-toluene.	Orange yellow.			4N, less than one-half extracted; N/64, almost all extracted.	Little extracted.	Chief part not extracted.	Larger part not extracted.	Chief part not extracted.
119		Benzene-azo-*β*-naphthylamin.	Orange yellow.			4N, almost all extracted; N/64, extracted.	Larger part not extracted.	Much extracted.	Chief part extracted.	Much extracted.
120		*O*-toluene-azo-*β*-naphthylamin.	Orange yellow.					More than ½ extracted.		More than ½ extracted.
121	666	Quinophthalon.	Yellow.				Little extracted.	Chief part not extracted.	Chief part not extracted.	Very little extracted.
122		Benzene-azo-*α*-naphthol.	Brown.				Very little extracted.	Very little extracted.	Very little extracted.	
123	59	*α*-Naphthalene-azo-*α*-naphthol.	Brown.			4N chief part extracted, N and below, extracted.	Chief part not extracted.	Larger part extracted.	Larger part extracted.	

124		*O*-toluene-azo-*o*-toluene-azo-α-naphthol.	Brownish red.			Practically all extracted, 4 N and below.	Chief part extracted.	Almost all extracted.	Almost all extracted.	Little extracted.
125	*11	Benzene-azo-β-naphthol.	Orange.				Chief part extracted.			½ or more extracted.
126	**49	*M*-xylene-azo-β-naphthol.	Orange red.							Chief part extracted.
127	60	α-Naphthalene-azo-β-naphthol.	Red							Almost all extracted.
128	*143	Benzene-azo-benzene-azo-β-naphthol.	Red.							
129	*	*O*-toluene-azo-*o*-toluene-azo-β-naphthol.								
130	31	*P*-nitrobenzene-azo-β-naphthol.	Orange.				Chief part not extracted.	Chief part extracted.		Little extracted.

Distribution of dyes between dilute sulphuric acid of varying concentrations and purified gasoline or petroleum spirit ("low b. p., sp. gr. 0.645). Statements refer in all cases to the amounts of the dyes extracted from 0.0025 – 0.005% solutions or mixtures in the acid, by an equal volume of the organic solvent. Ordinary concentrated sulphuric acid is approximately 35-normal.

Amino-azo-benzene (Aniline Yellow)...	Little or none extracted from acid of normality 2 or above.
Amino-azo-*o*-toluene (Spirit Yellow R)	½ or more extracted from 2-normal acid. Little or none extracted from acid 7-normal or stronger.
Dimethylamino-azo-benzene (Butter Yellow)	Little or none extracted from acid 7-normal or stronger.
Benzene-azo-α-naphthylamine.........	Similar to Aniline Yellow but much dye precipitated.
Amino-azo-α-naphthylamine...........	Little or none extracted from acid of normality above 5. At low normalities much dye precipitated.
Benzene-azo-β-naphthylamine (Yellow A. B.)	All or nearly all extracted from acid of normalities 10 and below. Little or none extracted at normalities 16 and above. Approximately ½ extracted from 12-normal acid.
O-toluene-azo-β-naphthylamine (Yellow O. B.)	All or nearly all extracted at normalities 10 and below. Little or none extracted at normalities of 18 and above. Approximately ½ extracted from 14-normal acid.
Benzene-azo-β-acetnaphthalide (Acetyl (Yellow A. B.)	Similar to Yellow A. B. but slightly less soluble relatively in the gasoline.
O-toluene-azo-β-acetnaphthalide.......	Similar to Yellow A. B.
P-toluene-azo-β-naphthylamine..	Similar to Yellow O. B.
M-xylene-azo-β-naphthylamine	Almost all extracted from acid of normality 12 or less. Little or none extracted at normalities 18 and above.
Benzene-azo-β-naphthol (Sudan I). . .	All or nearly all extracted from acid of normality below 16. Little or none extracted from 24-normal acid.
M-xylene-azo-β-naphthol (Sudan II)	All or nearly all extracted from acid 18-normal or below. Little or none extracted from 24-normal acid.
Benzene-azo-benzene-azo-β-naphthol (Sudan III)	Similar to Sudan II.
α-Naphthalene-azo-β-naphthol........	All or nearly all extracted from acid of normality 20 or less. Little extracted from 24-normal acid.
Benzene-azo-phenol..................	Less than ½ extracted from 3-normal acid. Little extracted at normalities 5 to 8. Little or none extracted at normalities above 10.
Benzene-azo-resorcinol (Sudan G).....	Very little extracted from acid 18-normal or stronger. Partially extracted at lower normalities.
α-Naphthalene-azo-α-a-naphthol (Sudan Brown)	Little or none extracted at normalities from 20 to 24. Dye soluble with difficulty both in gasoline and in dilute acid.
Benzene-azo-α-naphthol..............	Insoluble in gasoline.
Curcumine...........	Insoluble in gasoline.

Bixin..............................	Insoluble in gasoline. Bixin in solution in gasoline containing from 2 to 5% of chloroform is almost completely removed from the organic solvent by shaking with 24-normal acid; 16-normal acid removes very little. From a gasoline solution of a commercial annatto preparation 24-normal acid removed approximately ½.

Distribution of dyes between gasoline and fourth-normal hydrochloric acid.

Amino-azo-benzene (Aniline Yellow)........	Little extracted.
Dimethylamino-azo-benzene (Butter Yellow)	Chief part extracted.
Amino-azo-*o*-toluene (Spirit Yellow R)	Nearly all extracted.

IDENTIFICATION OF COLOURING SUBSTANCES

The colouring matters are usually obtained by the fractionation dissolved in various aqueous or organic solvents, but free from non-volatile substances. Neutral solutions suitable for certain tests are most easily obtained by evaporating to dryness and taking up the residue with water or other suitable solvent.

The general tests named below are easily made and are usually sufficient for the identification of dyes whose relative solubilities have been shown to some extent by the procedure of separation: reactions on the dyed fibre (or in solution) with acids and alkalies; behaviour on reduction and subsequent oxidation; the bromine test; reactions with nitrous acid. For ready comparison of colours of similar solubility, it is convenient to have tables of properties in which the arrangement is based on the solubility, and the reactions just named are described in this order on the following pages. Other test, or methods that are often useful are: reactions with potassium cyanide; reduction with subsequent separation and identification of the products; behaviour with acetic anhydride and sulphuric acid; behaviour with boric acid and concentrated sulphuric acid; spectroscopic and spectrophotometric examination; dyeing tests with cotton: and certain tests with metallic salts, used particularly for the natural colouring matters. These are discussed on pages 478–495. For convenience, the various commonly used identification tests for the natural colouring substances are described together on pages 495–497.

Reactions on Dyed Wool (or Silk) with Acids, Alkalies, Etc.

Small pieces or shreds of the dyed wool are distributed on a porcelain plate and thoroughly moistened with the reagents. The fibre must be dry or nearly so, and must have been dyed in a fairly pure solution of the colour, since colourless organic impurities may easily obscure the reaction.

TABLE 2.—BEHAVIOR OF DRY COLOURS OR OF DYED FIBRES WITH REAGENTS

[Numbers denoting permitted dyes are in boldfaced type; natural colours are in italics. Dyes common in foods are starred, three stars indicating those dyes most often found.]

No.	Colouring matter	Colour of sulphuric acid solution	Colour of dyed wool	Reactions of dyed wool with reagents: Concentrated hydrochloric acid	Concentrated sulphuric acid	10 % sodium hydroxide solution	Ammonia solution, sp. gr., 0.95
1	***462	Yellow.	Violet red.	Nearly decolorised.	Yellow.	Decolorised.	Decolorised.
2	434	Yellow.	Green.	Yellow.	Orange or pale brown.	Decolorised.	Decolorised.
3	435	Yellow.	Green.	Yellow.	Orange or pale brown.	Decolorised.	Decolorised.
4	*436	Yellow.	Greenish blue.	Yellow.	Orange or pale brown.	Little change (slightly darker).	Little change (slightly darker).
5	439	Yellow.	Greenish blue.	Yellow.	Orange or pale brown.	Yellow olive.	Redder.
6	491	Yellow.	Green.	Yellow.	Orange or pale brown.	Little change.	Little change.
7	440	Yellow.	Greenish blue.	Yellow.	Orange or pale brown.	Little change.	Little change.
8	**602	Deep blue.	Bluish gray to black.	Dull bluish.	Dull greenish.	Pale brownish red.	Pale reddish.
9	**108	Violet.	Red.	Bluish red.	Violet.	Brown.	Orange red.
10	***8	Brownish yellow.	Yellow.	Red.	Orange.	Little change.	No change.
11	9	Brownish yellow.	Yellow.	Red.	Orange.	Little change.	No change.
12	**89	Violet red.	Yellow.	Bluish red.	Bluish red.	Little change.	Little change.
13	692	Violet blue.	Blue.	Slightly darker.	Slightly darker.	Greenish yellow.	Greenish blue.
14	399	Reddish brown.	Orange yellow.	Yellowish, dull.	Reddish brown.	Orange.	Orange.
15	***106	Red violet.	Scarlet.	Red.	Violet red.	Yellowish brown.	Orange red.
16	107	Violet.	Red.	Slightly darker.	Violet to brownish.	Dull brownish.	Little change.
17	94	Yellow.	Yellow.	Slightly darker.	Slightly darker.	Little change.	Little change.
18	*398	Yellowish brown.	Yellowish green.	Yellowish.	Brownish yellow.	No change.	No change.
19	605	Green.	Red.	Little change.	Dark green.	Little change.	Little change.
20	604	Green.	Red.	Dull brown.	Dark green.	Little change.	Little change.
21	188	Greenish black.	Bluish black.	Greenish blue.	Olive green.	Black.	No change.
22	***14	Orange yellow.	Orange yellow.	Little change.	Orange.	Dull brownish red.	No change.
23	21	Red.	Violet red.	Orange red.	Brownish red.	Brownish red.	Little change.
24	318	Bluish green.	Blue.	Little change.	Greenish blue.	Red.	Redder.
25	20	Red.	Red.	Little change.	Darker.	Reddish brown.	Brown.
26	93	Violet	Violet red.	Little change.	Violet.	Slightly bluer.	Orange red.
27	***480	Brown.	Blue.	Paler.	Brown.	Pale reddish.	Almost decolorised.
28	*53	Violet red.	Scarlet.	Darker.	Violet red.	Brownish yellow.	No change.
29	*55	Red.	Scarlet.	Little change.	Little change.	Brownish yellow.	No change.
30	105	Violet.	Red.	Slightly bluer.	Reddish violet.	Dull brownish red.	Almost unchanged.
31	4	Pale yellow.	Yellow.	Nearly decolorised.	Very pale dull brown.	No change.	No change.
32	***706	Red orange.	Dull orange red.	Little change.	Little change.	Violet red.	Violet red.
33	56	Red.	Red.	Little change.	Little change.	Dull orange.	Little change.
34	*62	Blue.	Violet red.	Darker.	Violet.	Dull brown.	Little change.

35	**64	Violet.	Scarlet.	Violet red.	Violet.	Dull brown.	Little change.
36	*65	Blue.	Violet red.	Violet.	Blue.	Brown.	Little change.
37	**103	Violet.	Violet red.	Little change.	Violet.	Scarlet.	Scarlet.
38	139	Violet.	Brown.		Dark violet.	Darker.	Little change.
39	164	Blue.	Scarlet.				
40	**667	Orange red.	Yellow.	Slightly darker.	Brownish yellow.	Slightly paler.	Little change.
41	*169	Blue.	Red.	Blue.	Blue.	Dull violet red.	Little change.
42	163	Green.	Red.	Violet blue.	Green.	Dull violet.	Little change.
43	170	Blue.	Red.				
44	84	Yellow.	Orange yellow.	Orange.	Orange.	Dull orange red.	No change.
45	146	Reddish violet.	Red.	Blue.	Violet.	Brown.	No change.
46	287	Deep blue.	Reddish blue.	Darker.	Greenish blue.	Red.	Violet.
47	78	Violet red.	Red.	Brown.	Violet red.	Little change.	Little change.
48	***710	Violet.	Red.	Red.	Brown, dull.	Violet.	Violet.
49	*546	Orange.	Orange yellow.	Yellow.	Orange.	Violet.	Violet.
50	1	Almost colourless.	Yellow.	Decolorised.	Pale brownish.	Little change (slightly darker).	No change.
51	507	Orange.	Reddish violet.	Slightly yellower.	Orange.	Red.	Red.
52	328	Reddish violet.	Orange yellow.	Dull violet.	Brownish violet.	Orange red.	Orange red.
53	606	Dull green.	Orange.	Yellowish brown.	Dark green.	Little change.	Little change.
54	154	Violet blue.	Red.	Blue.	Blue.	Brownish violet.	Little change.
55	85	Violet.	Orange.	Violet.	Violet.	Red, dark.	Red, dark.
56	**13	Orange yellow.	Orange.	Orange red.	Orange.	Slightly darker.	No change.
57	***86	Red.	Orange.	Red.	Red.	Dull orange red.	No change.
58	97	Red.	Orange.	Red.	Red.	Browner.	Browner.
59	54	Orange red.	Orange.	Scarlet.	Scarlet.	Little change.	No change.
60	329	Reddish violet.	Yellow.	Blue.	Reddish violet.	Orange yellow.	No change.
61	137	Brown.	Brown.		Darker.	Darker.	
62	157	Bluish green.	Red.	Blue violet.	Green.	Brownish violet.	Little change.
63	*95	Violet.	Orange yellow.	Violet red.	Violet.	No change.	No change.
64	88	Violet.	Orange yellow.	Violet red.	Violet.	No change.	No change.
65	*92	Violet red.	Yellow.	Violet red.	Violet red.	Dull brown.	Little change
66	101	Blue.	Brown.	Reddish blue.	Blue.	Violet red.	Violet red.
67	102	Violet.	Red.	Red.	Violet.	Dull orange red.	No change.
68	483	Brownish yellow.	Orange red.	Orange yellow.	Dull orange yellow.	Little change.	Little change.
69	*510	Yellow.	Yellow.	Little change.	Little change.	Slightly redder, green fluor.	Little change, green fluor.
70	26	Orange yellow.	Orange yellow.	Orange.	Orange.	Orange red.	Orange.
71	220	Reddish violet.	Yellow.	Brown.	Violet red.	Red orange.	Orange yellow.
72	269	Reddish violet.	Yellow.	Brown.	Violet red.	Red orange.	Orange yellow.
73	512	Yellow.	Red.	Yellow orange.	Yellow orange.	No change.	No change.
74	515	Brownish yellow.	Violet red.	Yellow.	Brownish.	Scarlet.	Little change.
75	516	Brownish yellow.	Orange red.	Orange yellow.	Orange yellow.	No change.	No change.
76	517	Brownish yellow.	Violet red.	Orange yellow.	Orange yellow.	No change.	No change.
77	518	Brownish yellow.	Violet red.	Yellow.	Orange.	No change.	No change.
78	520	Orange.	Violet red.	Yellowish.	Orange.	No change.	No change.
79	521	Orange.	Violet red.	Yellowish.	Orange.	No change.	No change.

TABLE 2.—BEHAVIOR OF DRY COLOURS OR OF DYED FIBRES WITH REAGENTS.—(*Continued*)

No.	Colouring matter	Colour of sulphuric acid solution	Colour of dyed wool	Reactions of dyed wool with reagents: Concentrated hydrochloric acid	Concentrated sulphuric acid	10 % sodium hydroxide solution	Ammonia solution sp. gr., 0.95
80	*523	Orange.	Red violet.	Almost decolorised.	Orange.	No change.	No change.
81	2	Pale yellow.	Orange yellow.	Almost decolorised.	Pale brownish yellow.	No change.	No change.
82	*3	Pale yellow.	Yellow.	Almost decolorised.	Pale brownish yellow.	Little change.	Little change.
83	6	Very pale yellow.	Orange.	Almost decolorised.	Pale brownish yellow.	Dull orange red.	Slightly redder.
84	*634*	Orange red.	Orange.	Yellower.	Redder.	Violet.	Violet.
85	***707	Orange.	Yellow.	Red.	Reddish brown.	Orange.	Orange.
86	*10	Yellowish orange.	(Silk.) Orange yellow.	(Silk.) Orange yellow.	(Silk.) Brownish yellow.	(Silk.) Orange yellow.	(Silk.) No change.
87	*468	Orange yellow.	Violet.	Pale orange yellow.	Pale dull orange.	Decolorised.	Decolorised.
88	464	Yellow.	Bluish violet.	Pale orange yellow.	Pale dull orange.	Decolorised.	Decolorised.
89	438	Yellowish brown.	Green.	Orange yellow.	Brownish yellow.	Decolorised.	Paler.
90	433	Yellow.	Green.	Pale orange yellow.	Pale dull yellow.	Decolorised.	Decolorised.
91	442	Yellow.	Greenish blue.	Pale orange yellow.	Pale dull yellow.	Little change.	Little change.
92	476	Reddish brown.	Blue.	Little change.	Reddish brown.	Dull brown.	Little change.
93	240	Blue.	Reddish brown.	Blue.	Blue.	No change.	No change.
94	277	Blue.	Brownish red.	Blue.	Greenish blue.	Orange red.	Orange red.
95	562	Brownish red.	Violet blue.	Orange red.	Brownish red.	Green.	Blue.
96	658	Colourless.	Yellow.	Decolourized.	Almost decolorised.	Decolorised.	Paler.
97	496	Orange.	Red.	Orange yellow.	Yellow.	Paler.	Paler.
98	650	Yellowish green.	Blue.	Almost decolorised	Greenish, paler.	Pale violet.	No change.
99	639	Dark green.	Blue.	Violet blue.	Dull greenish.	Pale dull violet red.	Pale dull violet red.
100	*584	Green.	Red.	Greenish blue.	Green.	Red.	Red.
101	**448	Yellowish brown.	Violet red.	Yellowish brown.	Dull brown.	Decolorised.	Paler.
102	***425	Colourless.	Yellow.	Decolorised.	Nearly decolorised.	Decolorised.	Paler.
103	426	Colourless.	Yellow.	Decolorised.	Nearly decolorised.	Decolorised.	Paler.
104	***451	Yellow.	Violet.	Yellowish.	Yellowish.	Decolorised.	Almost decolorised.
105	452	Yellow.	Violet.	Yellowish.	Yellowish.	Decolorised.	Almost decolorised.
106	**427	Yellow.	Bluish green.	Almost decolorised.	Almost decolorised.	Decolorised.	Decolourized.
107	428	Yellow.	Green.	Almost decolorised.	Almost decolorised.	Decolorised.	Decolourized.
108	*197	Brown.	Reddish brown.	Redder, darker.	Browner.	Yellower.	Yellower.
109	201	Brown.	Reddish brown.	Redder, darker.	Browner.	Yellower.	Yellower.
110	17	Orange yellow.	Orange yellow.	Orange red.	Brownish red.	Slightly duller.	Yellower.
111	18	Orange yellow.	Orange yellow.	Red.	Brown.	Slightly duller.	Yellower.
112	505	Yellow.	Violet red.	Orange.	Yellow.	Slightly bluer.	Slightly bluer.
113	499	Yellow.	Bluish red.	Orange.	Yellow, pale.	Slightly bluer.	Slightly bluer.
114	***594	Yellowish brown.	Bluish red.	Orange.	Yellow.	Bluer.	Bluer.
115	502	Yellowish brown.	Bluish red.	Orange.	Yellow.	Slightly bluer.	Slightly bluer.

No.	Colouring matter	Colour of sulphuric acid solution	Colour of dyed silk	Reactions of dyed silk with reagents: Concentrated hydrochloric acid	Concentrated sulphuric acid	10 % sodium hydroxide solution	Ammonia solution, sp. gr., 0.95
116	**16. Dimethylamino-azo-benzene.	Yellow.	Orange yellow.	Red, bluish.	Orange yellow.	No change.	No change.
117	7. Amino-azo-benzene.	Yellow.	Orange yellow.	Brownish red.	Orange yellow.	Little change.	No change.
118	*. *O*-amino-azo-toluene.	Orange yellow.	Orange yellow.	Dull orange.	Orange yellow.	Little change.	No change.
119	Benzene-azo-β-naphthylamin.	Reddish violet.	Yellowish brown.	Red.	Violet.	Little change.	No change.
120	Ortho-toluene-azo-β-naphthylamin.	Violet.	Yellowish brown.	Red.	Violet.	Little change.	No change.
121	666. Quinophthalon.	Reddish brown.	Yellow.	Orange yellow.	Brownishyellow.	Orange yellow.	Little change.
122	Benzene-azo-naphthol.	Violet.	Orange.	Violet red.	Violet.	Deep red.	Deep red.
123	59. Naphthalene-azo-α-naphthol.	Greenish blue.	Brown.	Bluish violet.	Greenish blue.	Bluish red.	Bluish red.
124	Ortho-tolueneazo-α-toluene.	Green.	Red.	Blue.	Green.	Reddish blue.	Reddish blue.
125	*11. Benzene-azo-β-naphthol.	Red.	Orange.	Orange red.	Red.	Redder.	Little change.
126	**49. *M*-xylene-azo-β-naphthol.	Violet red.	Orange red.	Red.	Violet red.	Little change.	No change.
127	60. α Naphthalene-azo-β-naphthol.	Bluish violet.	Bluish red.	Slightly bluer.	Dull blue.	Browner and paler.	Slightly paler.
128	*143. Benzene-azo-benzene-azo-β-naphthol.	Green.	Red.	Violet, then brown.	Green.	Violet red.	Little change.
129	*. *O*-toluene-azo-*o*-toluene-azo-β-naphthol.	Green.	Violet red.	Violet blue.	Bluish green.	Violet red.	Little change.
130	31. *P*-nitrobenzene-azo-β-naphthol.	Violet red.	Brownish red.	Orange red.	Violet red.	Violet blue.	No change.

With concentrated solution of stannous chloride in hydrochloric acid the fiber is decolorised in all cases except that of No. 666.

The dyeings obtained from colours in food products are necessarily variable in depth and usually paler than those used as a basis for standards. With the very small amounts of colour available it is impossible to make dyeings to any convenient standard depth, and descriptions can only indicate in a general way what may be expected. Not only will the appearance of the dyed wool under the influence of different reagents vary somewhat with the concentration of the dye present, but the shade of the dry fibre also may vary with the concentration of the dye. For example, dyeings from some of the oranges are almost yellow when only a little colour is present, but are a much redder shade when more dye is used.

Colour changes similar to those taking place on dyed fibers are produced in most cases by the given reagents in solutions of the dyes, and the conditions are under much more exact control. So in some cases it is advantageous to compare the solution of the dye under examination with solutions of known colours, all being brought as nearly as possible to the same dye concentration, and to the same acid or alkali normality. An exact statement of shade can be given best by spectrophotometric data under prescribed conditions of temperature concentration.

Reactions with Sodium Hydrosulphite

The neutral colour solution is treated with a few particles of powdered sodium hydrosulphite,[1] conveniently dropped in from a small spatula. If no colour change is seen at once, the mixture is warmed somewhat and more reagent added, carefully avoiding excess, however. If reduction, shown by disappearance of the colour, takes place, the solution is thoroughly shaken with air, and should this not bring back the dye, it is warmed and allowed to stand a few minutes. Finally, if remaining practically colourless, a little powdered potassium persulphate is dropped in. A slightly yellowish or brownish tint produced by air or especially by the potassium persulphate is disregarded.

[1] Hydrosulphite and persulphate are the reagents advocated by Green, Yeoman, and Jones, *J. Soc. Dyers & Col.*, 1905, **9**, 236; also Green, *Identification of Dye Stuffs*, Leeds (1913).

TABLE 3.—BEHAVIOUR OF COLOURS WHEN TREATED WITH REDUCING AGENTS FOLLOWED BY OXIDISING AGENTS

[Numbers denoting dyes permitted in the U. S. A. are in boldfaced type; natural colours are in italics. Dyes common in foods are starred, three stars indicating those most often found. Statements apply in general to 0.01 per cent. solutions.]

No.	Colouring matter	With sodium hydrosulphite	Colouring matter	Reduction product with air or potassium persulphate
1	*462	Almost decolorised.	*462	Colour restored.
2	434	Almost decolorised.	434	Colour partially restored.
3	**435**	Almost decolorised.	**435**	Colour partially restored.
4	*436	Becomes paler very slowly.	*436	...
5	439	Paler, slowly.	439	Colour restored.
6	491	Almost decolorised.	491	Some colour returns.
7	440	Decolorised.	440	Colour restored.
8	**602	Pale olive.	**602	Colour restored.
9	**108	Decolorised.	**108	Remains colourless or nearly so.
10	***8	Decolorised.	***8	Remains colourless or nearly so.
11	9	Decolorised.	9	Remains colourless or nearly so.
12	**89	Decolorised.	**89	Remains colourless or nearly so.
13	**692**	Decolorised.	**692**	Colour restored.
14	399	Not decolorised.	399	...
15	***106	Decolorised.	***106	Remains colourless or nearly so.
16	107	Decolorised.	**107**	Remains colourless or nearly so.
17	**94**	Decolorised.	**94**	Remains colourless or nearly so.
18	*398	Decolorised.	*398	Remains colourless or nearly so.
19	605	Pale orange.	605	Colour restored.
20	604	Pale orange.	604	Colour restored.
21	198	Decolorised.	198	Remains colourless or nearly so.
22	***14	Decolorised.	***14	Remains colourless or nearly so.
23	21	Decolorised rather slowly.	21	Remains colourless or pale brownish.
24	318	Bluer; then decolorised.	318	Remains colourless or nearly so.
25	20	Decolorised.	20	Remains colourless or nearly so.
26	93	Decolorised.	93	Remains colourless or nearly so.
27	***480	Much paler.	***480	Colour restored.
28	*53	Decolorised.	*53	Remains colourless or nearly so.
29	*55	Decolorised.	*55	Remains colourless or nearly so.
30	105	Decolorised.	105	Remains colourless or nearly so.
31	**4**	Decolorised.	**4**	Remains colourless or nearly so.
32	***_706_	Not decolorised.	***_706_	...
33	**56**	Decolorised.	**56**	Remains colourless or nearly so.
34	*62	Decolorised.	*62	Remains colourless or nearly so.
35	**64	Decolorised.	**64	Remains colourless or nearly so.
36	*65	Decolorised.	*65	Remains colourless or nearly so.
37	**103	Decolorised.	**103	Remains colourless or nearly so.
38	139	Decolorised.	139	Remains colourless or nearly so.
39	164	Decolorised	164	Remains colourless or nearly so.
40	**667	Not changed.	**667	...
41	*169	Bluer; then decolorised.	*169	Remains colourless or nearly so.
42	163	Decolorised.	163	Remains colourless or nearly so.
43	170	Bluer; then decolorised.	170	Remains colourless or nearly so.
44	84	Decolorised.	84	Remains colourless or nearly so.
45	146	Bluer; then decolorised.	146	Remains colourless or nearly so.
46	287	Slowly decolorised.	287	Remains colourless or nearly so.
47	78	Slowly decolorised.	78	Remains colourless or nearly so.
48	***_710_	Decolorised, nearly.	***_710_	Colour restored.
49	*546	Not changed.	*546	...
50	1	Decolorised.	1	Not restored.
51	507	Not changed.	507	...
52	328	Decolorised.	328	Remains colourless or nearly so.
53	606	Pale yellow.	606	Colour restored.
54	154	Bluer; then decolorised.	154	Remains colourless or nearly so.
55	**85**	Decolorised.	**85**	Remains colourless or nearly so.

TABLE 3.—BEHAVIOUR OF COLOURS WHEN TREATED WITH REDUCING AGENTS FOLLOWED BY OXIDISING AGENTS.—(*Continued*)

No.	Colouring matter	With sodium hydrosulphite	Colouring matter	Reduction product with air or potassium persulphate
56	**13	Decolorised.	**13	Remains colourless or nearly so.
57	***86	Decolorised.	***86	Remains colourless or nearly so.
58	97	Decolorised.	97	Remains colourless or nearly so.
59	54	Decolorised.	54	Remains colourless or nearly so.
60	329	Decolorised.	329	Remains colourless or nearly so.
61	139	Decolorised.	139	Remains colourless or nearly so.
62	157	Decolorised.	157	Remains colourless or nearly so.
63	*95	Decolorised.	*95	Colourless or slightly yellow.
64	88	Decolorised.	88	Colourless or slightly yellow.
65	*92	Decolorised.	*92	Remains colourless or nearly so.
66	101	Decolorised.	101	Remains colourless or nearly so.
67	102	Decolorised.	102	Remains colourless or nearly so.
68	483	Decolorised.	483	Colour restored.
69	*510	Much paler.	*510	Colour restored.
70	26	Decolorised.	26	Remains colourless or nearly so.
71	220	Decolorised.	220	Remains colourless or nearly so.
72	269	Decolorised.	269	Remains colourless or nearly so.
73	512	Much paler (with excess).	512	Colour restored.
74	515	Much paler (with excess).	515	Colour restored.
75	516	Much paler (with excess).	516	Colour restored.
76	517	Much paler (with excess).	517	Colour restored.
77	518	Much paler (with excess).	518	Colour restored.
78	520	Much paler (with excess).	520	Colour restored.
79	521	Much paler (with excess).	521	Colour restored.
80	*523	Much paler (with excess).	*523	Colour restored.
81	2	Decolorised.	2	Remains colourless or nearly so.
82	*3	Decolorised.	*3	Remains colourless or nearly so.
83	6	Dark; then pale.	6	Pale reddish.
84	*634*	(Alk. sol.) red, slowly.	*634*	Colour restored.
85	****707*	Not reduced.	****707*	. . . .
86	*10	Decolorised.	*10	Remains colourless or nearly so.
87	*468	Decolorised.	*468	Colour restored.
88	464	Decolorised.	464	Colour restored.
89	438	Almost decolorised.	438	Colour restored.
90	**433	Paler.	433	Greener.
91	442	Paler, slowly.	442	Restored.
92	476	Not readily reduced.	476	. . . .
93	240	Almost decolorised.	240	Remains colourless or nearly so.
94	277	Browner; then colourless.	277	Remains colourless or nearly so.
95	562	(Alk. sol.), yellow.	562	Colour restored.
96	658	No change.	658	. . . .
97	496	Almost decolorised.	496	Colour largely restored.
98	650	Decolorised.	650	Colour restored.
99	639	Decolorised.	639	Colour restored.
100	*584	Decolorised.	*584	Colour restored.
101	**448	Decolorised.	**448	Colour restored.
102	***425	Not decolorised.	***425	. . . .
103	426	Not decolorised.	426	. . . .
104	***451	Decolorised.	***451	Colour restored.
105	452	Decolorised.	452	Colour restored.
106	**427	Decolorised.	**427	Colour restored.
107	428	Decolorised.	428	Colour restored.
108	*197	Almost decolorised.	*197	Colourless or nearly so.
109	*201	Almost decolorised.	*201	Colourless or nearly so.
110	17	Decolorised.	17	Remains colourless or nearly so.
111	18	Decolorised.	18	Remains colourless or nearly so.
112	505	Not decolorised.	505	. . . .
113	499	Not decolorised.	499	. . . .
114	***504	Not decolorised.	***504	. . . .
115	502	Not decolorised.	502	. . . .

In alkaline solutions the reductions take place more slowly. Ammoniacal solutions of Naphthol Yellow S become rose red on the addition of a few particles of sodium hydrosulphite, the coloration

gradually fading as further reduction takes place. Other polynitro dyes behave similarly.

THE BROMINE TEST

This test (*U. S. Dept. Agr. Bull.*, 448) is valuable for quickly testing the colour solutions obtained in the fractionation. The free acid need not be removed; though, as described in detail below, minor differences exist, depending on whether the solutions are practically neutral or markedly acid. They must not be alkaline and should be free from foreign material, though dissolved amyl alcohol does not interfere with the test. With the oil-soluble dyes, the oxidation may be made in acetic acid of from 50 to 80% strength.

The chief practical use of the test is for the detection of the azo and the azine dyes, especially when in admixture with natural colouring matters. It provides the simplest means for the identification of the "first component" of the azo colours, for which, of course, reduction methods may also be applied. The test is made as follows: About 5 c.c. of the dye solution (preferably of concentration in the neighborhood of from 0.005 to 0.01% are treated with bromine water (1%) added drop by drop until a little more has been used than is required to destroy the dye. A few drops of 3% hydrazine sulphate solution are then added and the mixture divided into two portions. To one is first added a few drops of alcoholic alpha-naphthol solution, then excess of sodium carbonate; to the other, sodium carbonate only. With azo compounds sodium formate may be substituted for the hydrazine salt.

The reactions obtained are referred to classes as follows:

Class A-Azo Dyes.—These yield on oxidation in acid or neutral solutions a diazo compound corresponding to the "first component" of the dye.[1] The azo group remains attached to the non-hydroxylated or non-aminated residue, and it is noteworthy that with Chrysophenine (No. 329), the one azo colour described in the table containing neither hydroxyl nor amino groups, the usual reaction is not obtained. With dis-azo colours of the type of Cotton Scarlet, $C_6H_5N_2C_6H_4N_2C_{10}H_5OH\ (SO_3Na_2)_2$, the azo group between the two non-hydroxyl-containing residues is not readily attacked, so that a diazo compound is formed. With dyes of class A the solution

[1] For the action of halogens on azo compounds, see M. Schmidt, *J. prakt. Chem.*, 1912, **84**, 235. Oxidation by lead peroxide, Lauth, *Bul. Soc. Chim.*, 1891, **6**, 111, 94; by nitric acid, O. Schmidt, *Ber.*, 1905, **38**, 3201, 4022. See also Meldola, *Proc. Chem. Soc.*, 1894, **10**, 118, and *Trans. Chem. Soc.*, 1889, **55**, 608, and **65**, 1894, 841.

becomes colourless, pale yellow, or pale orange on addition of bromine. After addition of the hydrazine sulphate the solution is colourless or pale brownish or pinkish, a tendency to show a slight coloration being more marked, the more nearly neutral the solution. Addition of sodium carbonate alone produces no marked coloration, but α-naphthol, followed by the carbonate, gives a pronounced colour. It is advisable to add some ether to the coloured mixture and shake; since if the first component of the original dye was an unsulphonated amine (indicated by "e" in the table) the new colouring matter formed will be taken up by ether from the alkaline mixture, giving usually an orange solution which, on being poured off and treated with a large excess of concentrated hydrochloric acid, becomes in most cases, violet or blue. If the new dye is sulphonated (indicated by "w"), it will not be extracted by the ether from the mixture. When desired, the α-naphthol derivative, after separation by a suitable solvent, may be dyed on wool or silk and further identified by the ordinary spot tests with acids and alkalies. See tables for solubilities and reactions of Orange I (No. 85), Fast brown N (No. 101), benzene-azo-α-naphthol, and α-naphthalene-azo-α-naphthol (serial numbers in tables: 55, 66, 122, 123, respectively.

Other compounds, such as α-naphthylamine readily coupling with diazo compounds, may, of course, be used instead of the α-naphthol, sodium acetate being substituted for the sodium carbonate when an amino compound is employed. With the simple monazo colours, the reaction seems almost quantitative. With benzidine dyes it takes place least smoothly.

Class AA-Azo dyes react like the preceding class in solutions that contain a considerable amount of free hydrochloric acid (perhaps N/2 or above). In neutral solutions other oxidation reactions take place, so that a more or less strong coloration is produced on treatment with sodium carbonate without previous addition of the naphthol. Crystal Scarlet (No. 64), Bordeaux B (No. 65), and Palatin Red (No. 62) give strong blue colorations; Naphthol Black (No. 88) and Amaranth (No. 107) a less intense blue colour. Azorubin (103) gives a purple changing to bluish red, but not very intense. Except for the first three dyes named, the colorations are considerably less intense than the original dyes, and the reaction much less trustworthy and valuable than the smooth, almost quantitative, reaction in acid solutions. When treated with bromine in solutions

containing sodium carbonate (one-fourth normal), Nos. 62, 64 and 65 are bleached, becoming intensely blue on addition of hydrazine sulphate.

Class B.—Azine derivatives,[1] etc. On treatment with bromine in neutral or acid solutions, the colour is readily bleached, but is restored on adding hydrazine sulphate. Sodium carbonate, and alpha-naphthol plus sodium cabornate produce no change other than that shown by the original dye solution on treatment with alkali. A few dyes of Classes A and AA when oxidised in neutral solutions tend to show a rather marked coloration on adding the hydrazine sulphate. However, with the typical members of Class B, the dye may be bleached and restored a number of times by careful alternate addition of the two reagents, the bromine apparently forming a nearly colourless compound reconverted into the original dye on addition of the hydrazine sulphate.

Class C.—Colours giving precipitates at dilutions as high as 0.01 per cent. This class includes most of the basic dyes.

Class D.—Colours showing marked changes in tint in neutral or very faintly acid solution on addition of bromine.[2] The colorations are usually produced by a mere trace of the halogen and destroyed by excess, and the reactions are consequently not very dependable or valuable. With many of the dyes of this type containing alkylated amino groups the colour change would seem to be due to elimination of alkyl radicals. In general, with dyes of this group the colouring matter, while readily altered, is completely broken down by bromine only with difficulty. On addition of hydrazine sulphate no change takes place other than that due to removal of the excess of the coloured halogen. (Chlorine water may be used if preferred.) The coloration with alpha-naphthol and sodium carbonate is identical with that produced by sodium carbonate alone. With most of the yellow colouring matters of this group it is brownish. With most of the others it is ill-defined and is probably often produced by a small fraction of the dye that has escaped destruction by bromine. In acid solutions these dyes are merely destroyed by bromine in most cases, the results being as given for E.

[1] Quinophthalone by treatment with bromine first forms the unstable colourless addition product containing two atoms of bromine in the molecule (Eibner and Lange, Annalen 1901, **315**. For action on azine dyes compare Vaubel, *J. prakt. Chem.* 1896, **54**, 289.

[2] According to Heumann, Die Anilinfarben, vol. 1, p. 41, Malachite Green solution, on oxidation with lead peroxide and acetic acid, becomes violet and then contains the salt of diaminotriphenylcarbinol. Compare further Vaubel, *J. prakt. Chem.* 1894, **50**, 347.

Class E.—Dyes in this class are similar to those in Class D, but show no colour changes other than bleaching, on addition of bromine.

TABLE 4.—THE BROMINE TEST: CLASSIFICATION OF COLOURS ACCORDING TO REACTIONS OBTAINED

[Numbers denoting dyes permitted in the U. S. are in bold-faced type; natural colours are in italics. Dyes common in foods are starred, three stars indicating those often found. Statements apply in general to 0.01 per cent. solutions.]

No.	Colouring matter	Class	No.	Colouring matter	Class	No.	Colouring matter	Class	No.	Colouring matter	Class
1	*462	D or E.	33	**56**	A (e).	65	*92	A (w).	87	*468	E.
2	434	D.	34	*62	AA (e).	66	101	A or AA (w).	88	464	E.
3	**435**	D.	35	**64	AA (e).	67	102	A (w).	89	438	D.
4	*436	D.	36	*65	AA (e).	68	483	E.	90	**433**	D.
5	439	E.	37	**103	A or AA (w).	69	*510	([3])	91	442	E.
6	491	D or E.	38	139	A or AA (w).	70	26	A (e).	92	476	E.
7	440	E.	39	164	A (w).	71	220	A.[1]	93	240	A.[5]
8	**602	E (B).[1]	40	**667	B.	72	269	A.[1]	94	277	A.
9	**108	A (w).	41	*169	A (w).	73	512	E.	95	562	B.
10	***8	A (w).	42	163	A (w).	74	515	E.	96	658	E.
11	9	A (w).	43	170	A (w).	75	516	E.	97	496	D or C.
12	**89	A (w).	44	84	A (w).	76	**517**	E.	98	650	E or C.
13	**692**	E.	45	146	A (e).	77	518	E.	99	639	E or D.
14	399	A (w).	46	287	A.[1]	78	520	E.	100	*584	B or C.
15	***106	A (w).	47	78	A.[2]	79	521	E.	101	**448	E or D.
16	**107**	AA or A (w).	48	****710*	E.	80	*523	E.	102	***425	E or C.
17	**94**	A (w).	49	*546	B.[1]	81	2	E.	103	426	E or C.
18	*398	E.	50	1	E.	82	*3	E.	104	***451	E or C.
19	605	B.	51	507	D.	83	6	E.	105	452	E or C.
20	604	B.	52	328	A (w).	84	*534*	B.	106	**427	D or C.
21	188	AA (w).	53	606	B.	85	****707*	E.	107	428	D or C.
22	***14	A (e).	54	154	A (e).	86	*10	A (e).[4]	108	*197	A or C.
23	21	A (e).	55	**85**	A (w).				109	*201	A or C.
24	318	A.[1]	56	**13	A (e).				110	17	A or C(e).
25	20	A (e).	57	**86	A (w).				111	18	A or C(e).
26	93	A or AA (w).	58	97	A (w).				112	505	D or C.
27	***480	D.	59	54	A (e).				113	499	D or C.
28	*53	A (e).	60	329	E.				114	***504	D or C.
29	*55	A (e).	61	139	A (w).				115	502	D or C.
30	105	A (w).	62	157	A (e).				([4])		
31	4	E.	63	*95	A (w).				. .		
32	****706*	E.	64	88	A (w).				. . .		

[1] Imperfectly.
[2] Some alcohol should be added before the alpha-naphthol.
[3] Gives eosine.
[4] Of the oil-soluble dyes given in the other tables all belong to type A except Quinophthalon, which in 60 per cent. acetic acid shows reaction indicated under B.
[5] Very imperfectly.

Class F.—Halogenated fluoresceine derivatives and similar compounds. These dyes are very resistant to bromine. The non-fluorescent iodine compounds tend to become yellower in shade and to develop a green fluorescence, probably due to partial substitution of iodine by bromine.

REACTIONS WITH NITROUS ACID

By treatment with nitrous acid in dilute solution most of the common coal tar dyes used in food colouring are not readily affected.

A considerable number, however, show marked changes, because of diazotization of free amino groups, of formation of nitroso compounds, or of direct oxidation.

When diazo compounds are formed, they may be further coupled by the usual method of adding the mixture to an alkaline solution of one of the naphthols, or of a naphthol sulphonic acid. B. C. Hesse has pointed out that the two acid yellows (No. 8 and No. 9) can be distinguished by the use of alpha-naphthol, No. 9 giving in alkaline solution a red compound; No. 8 one which is intensely blue.

In the test described below the mixture is treated first with nitrous acid, then with hydrazine sulphate (*J. Assoc. Off. Agr. Chem.*, 1922, **6**, 25. *U. S. Dept. Agr. Bull.*, 448). The hydrazine sulphate serves to destroy the excess of nitrous acid, so that the naphthol solution (or resorcinol if preferred) may be added directly, and the coupling then brought about by addition of alkali. The new dye formed may also be separated readily, if desired, by acidifying and shaking out with a suitable solvent. In the case of only one dye in the table, Safranine (No. 584), does the diazo compound appear to be changed very rapidly by the hydrazine sulphate.

The nitroso compounds formed from Nos. 95 and 88, are decomposed by the hydrazine salt, the original colour of the acid solution being restored.

The test is carried out as follows: The solution of the colour at ordinary temperature is made slightly acid by the addition of two or three drops of concentrated hydrochloric acid and one or two drops of 7 per cent. sodium nitrite solution are added. With blue or green dyes, where oxidation changes may take place, the mixture may be allowed to stand for a few minutes at this stage; but with other colours about 1 c.c., or an excess, of 3 per cent. hydrazine sulphate solution is added at once. The mixture is allowed to stand one-half or one minute to permit complete destruction of the excess of nitrous acid; then it is divided and a few drops of alpha-naphthol solution are added to one portion. Both portions are then made strongly alkaline with sodium carbonate, the one not containing alpha-naphthol serving as a check to show if any coupling has taken place.

Resorcinol is in some cases a better reagent than alpha-naphthol for detecting diazo compounds formed by the sodium nitrite in this test. For example, the Benzo Blues (obtained by coupling H-acid in alkaline solution with tetrazodiphenyl or one of its homologues)

show no striking colour changes in this test, since both the diazo compound from the dye and the product obtained by coupling it with naphthol form violet solutions. With resorcinol, however, a green solution is obtained.

TABLE 5.—BEHAVIOUR OF COLOURS WHEN TREATED WITH SODIUM NITRITE

[° Indicates that no colour changes take place other than those produced by the acid or alkali.]

462.—With sodium nitrite, blue; then colourless; after making alkaline in the presence of alpha-naphthol, orange.
434°, 435°, 436°.—Attacked very slowly by nitrous acid.
439.—Becomes yellow with sodium nitrite.
491.—Becomes violet with sodium nitrite (rather slowly).
440°, 602°, 108°.
8.—With sodium nitrite, much paler; after adding alpha-naphthol and *excess* of sodium carbonate, intensely blue.
9.—With sodium nitrite, much paler; after adding alpha-naphthol and *excess* of sodium carbonate, red.
89.—Red solution becomes yellow with sodium nitrite; on addition of hydrazine sulphate, red again.
692.—With sodium nitrite, slowly oxidised to the yellow isatin derivative.
399°, 106°, 107°, 94°.
398.—With sodium nitrite, brown.
605°, 604°, 188°, 14°.
21.—With sodium nitrite, slightly darker; with alpha-naphthol and sodium carbonate, dull greenish black.
318.—With sodium nitrite, paler and redder.
29°, 93° (480°.—Slowly attacked by nitrous acid); 53°, 55°, 105°, 4°, 706°, 56°, 62°, 64°, 65°, 103°, 139°, 164°, 667°, 169°, 163°, 170°.
84.—With sodium nitrite, redder.
146°, 287°, 78°, 710°, 546°, 1°.
507.—With sodium nitrite, bluer.
328°, 606°, 154°.
85.—With sodium nitrite, paler.
86°, 54°, 13°, 97°, 319°, 129°, 157°.
95.—Crimson solution becomes yellow with sodium nitrite; on addition of hydrazine sulphate, red again.
88.—Crimson solution becomes yellow with sodium nitrite; on addition of hydrazine sulphate, red again.
92°.
101.—Paler with sodium nitrite.
102°, 483°, 510°, 26°.
220, 229.—Slightly paler with sodium nitrite.
512°, 515°, 516°, 517°, 518°, 520°, 521°, 523°, 2°, 3°, 6°, 534°, 707°, 10°, 468°, 464°, 438°, 433°, 442°, 476°, 240°, 277° (562°, scarcely attacked; in 50 per cent. acetic acid, behaves with nitrous acid as with bromine in the bromine test); 658°, 496°, 650°, 639°.
584.—With sodium nitrite, blue; rather rapidly becomes red again on addition of hydrazine sulphate.
448.—Wine-red on diazotisation, addition of hydrazine sulphate, alpha-naphthol and sodium carbonate; with sodium nitrite in acetic acid solution, first blue, then colourless.
425°, 426°, 451°, 452°.
427.—Reddish with sodium nitrite.
197°, 201°.

17.—With sodium nitrite, paler; after addition of sodium carbonate, naphthol, etc., somewhat redder.

18. With sodium nitrite, paler; after addition of sodium carbonate, naphthol, etc., somewhat redder.

505°, 499°, 504°, 502°.—May appear bluer when the alcoholic alpha-naphthol solution is added.

16°.—Slowly destroyed by nitrous acid.

7.—Paler with sodium nitrite; after addition of other reagents, red.

Amino-azo-toluene.—As stated above for 7.

Benzene-azo-β-naphthylamine, Ortho-toluene-azo-β-naphthylamine.—These compounds are almost insoluble in aqueous liquids. As ortho-amino-azo derivatives, they are not readily diazotized or coupled.

REACTIONS WITH POTASSIUM CYANIDE

With the common monazo dyes, the bromine oxidation will provide for an identification of the "first component" of the colour, *i. e.*, the radical not containing the hydroxyl or amino groups. The other radical, usually containing hydroxyl or amino groups ortho to the azo junction, is identified with much more difficulty in most cases. Since the two water-soluble ortho-azo dyes permitted in foods by the U. S. federal regulations are both derived from 2-naphthol-3-6-disulphonic acid as second component, the reaction discovered by Lange (*Deutsches Reichs Patent* No. 189,035), according to which derivatives of this acid are attacked on boiling with potassium cyanide and the 3-sulphonic acid group replaced by cyanogen, is useful for distinguishing and separating isomeric dyes.

The test may be made as follows: About 10 c.c. of the neutral colour solution are treated with 1 c.c. of 20 per cent. potassium cyanide solution and 1 c.c. of 20 per cent. ammonium chloride solution, heated in a test tube in a boiling water bath for from five to eight minutes and then quickly cooled. The reactions obtained with certain dyes are shown in the table. The test requires some care, and blanks with known dyes should be carried through at the same time in all cases.

The results with a number of common azo dyes are shown in the Table 6, the derivatives of 2-naphthol-3-6-disulphonic acid forming new dyes of markedly different solubilities, corresponding to the fact that they contain one less sulphonic acid group. By warming with the cyanide solution for a considerable period of time further reactions easily take place, derivatives of 2-naphthol-3-6-disulphonic acid and 2-naphthol-6-8-disulphonic acid being especially unstable.

The common nitro dyes are changed by warming with cyanide solution, becoming brownish or reddish (compare formation of isopurpuric acid from trinitrophenol).

TABLE 6.—BEHAVIOUR OF COLOURS WITH CYANIDE SOLUTION

Dye	"Second component" of dye	Behavior with cyanide solution
108	Naphthol trisulphonic acid (2–3–6–8).	Warmed 8 minutes, dye almost completely destroyed with production of orange and yellow substances. Warmed until dark red (1–2 minutes), strongly acidified, and washed with 2 N HCl, practically no colour is removed (3–4 washings); then washed with N/4HCl, a bluish red dye is readily removed.
Azorubin S. G.		Apparently unchanged by cyanide.
106	Naphthol disulphonic acid (2–6–8).	Dye is not changed in solubility, although on long warming much colour is destroyed. The cyanide mixture may be acidified with 5 c.c. concentrated hydrochloric acid, and shaken out with 10 c.c. of amyl alcohol. On separating the alcohol, and washing 4 or 5 times with fourth-normal hydrochloric acid, nearly all of the dye will be taken out by the dilute acid.
107	Naphthol disulphonic acid (2–3–6).	Dye is changed into a cyan-derivative similar in solubility to other disulphonated monazo dyes. The cyanide mixture is pale brown and when treated as stated under New Coccin (106), almost all colouring matter remains in the amyl alcohol. On long heating of the cyanide mixture the cyan-derivative may be completely destroyed, further reactions taking place.
14	Naphthol disulphonic acid (2–6–8).	Dye unchanged. Cyanide mixture, when acidified with 1 c.c. glacial acetic acid and shaken with 5 to 10 c.c. amyl alcohol, gives up little couloring matter to the latter.
15	Naphthol disulphonic acid (2–3–6).	Dye changed into a cyan-derivative similar in solubility to the other monosulphonated monazo dyes. The cyanide mixture is pale brownish, and when treated as described under Orange G (14) gives up most of its colouring matter to the alcohol.
20	Dioxynaphthalene disulphonic acid (1–8–3–6).	As stated for 14.
21	Aminonaphthol disulphonic acid (1–8–3–6).	As stated for 14.
52	Naphthol disulphonic acid (1–4–8).	As stated for 14.
53	Naphthol disulphonic acid (1–3–6).	As stated for 14.
55	Naphthol disulphonic acid (2–3–6).	As stated for 15.
56	Naphthol disulphonic acid (2–3–6).	As stated for 15.
62	Naphthol disulphonic acid (1–3–6).	As stated for 14.
64	Naphthol disulphonic acid (2–6–8).	As stated for 14.
65	Naphthol disulphonic acid (2–3–6).	As stated for 15.

REDUCTION WITH SUBSEQUENT SEPARATION AND IDENTIFICATION OF THE REDUCTION PRODUCTS

The separation of the amino compounds formed by the reduction of azo dyes must usually be effected with immiscible solvents in dealing with the small quantities from coloured foods and drugs. The direct cotton dyes obtained by coupling diazotized benzidine or homologues with sulphonated naphtholic compounds do not react smoothly in the bromine test, so that reduction methods are

especially useful for their examination. The neutral or slightly acid solution of the dye is treated with just enough titanium trichloride, sodium hydrosulphite or stannous chloride solution to decolorise it, then made alkaline with sodium hydroxide and shaken with ether. The washed ether solutions may be evaporated to dryness and the neutral residue tested for benzidene with potassium dichromate, or better, it may be dissolved in dilute hydrochloric acid, the solution (at room temperature) treated with a drop of dilute sodium nitrite solution, and the mixture poured into an alkaline solution of sodium a-naphthol-2-sulphonate, the azo dye formed being subsequently extracted and further examined if desired. The dye formed from benzidine is violet red in alkaline solution whilst those from aniline, the toluidines and the naphthylamines are orange or orange red. (*J. Assoc. Off. Agr. Chem.*, 1922, **6**, 19.) If the solution obtained by reducing an azo dye in the manner just described is treated directly with sodium nitrite, etc., according to the procedure outlined on page 488 a coloration identical with that given by the bromine test will usually be obtained. The amino-naphthol or diamine does not diazotise readily while the amine forming the "first component" gives the same dye as is formed by the bromine test. The formation of the diazo compound by the use of bromine is usually preferable when nitro groups are present.

For the identification of the simpler azo dyes by reduction, separation of the reduction products and characterisation of these by coupling with diazo compounds, by condensation with nitrosodimethylaniline, and by diazotisation, see especially Witt, Ber. 1886, **19**, 1719, and 1888, **21**, 3468. Properties of the various amines, aminophenols, and their sulphonic acids are summarised by Heumann (Freidlander, Schultz), Die Anilinfarben. Braunschweig, 1888–1906.

BEHAVIOUR WITH ACETIC ANHYDRIDE AND CONC. SULPHURIC ACID

Some of the oil-soluble colouring matters show characteristic colour reactions when the freshly prepared solutions in acetic anhydride are treated with a few drops of sulphuric acid. The colorations produced by addition of 2 drops (01. c.c.) concentrated sulphuric acid to 5 c.c. of 0.005% solutions of various oil soluble colouring matters are described below. These colorations are permanent for 20 minutes or more unless otherwise stated.

COLORATIONS PRODUCED BY ADDITION OF 2 DROPS (0.1 C.C). CONCENTRATED SUPLHURIC ACID TO 5 C.C. OF 0.005% SOLUTIONS OF OIL-SOLUBLE COLOURING MATTERS IN ACETIC ANHYDRIDE

Dye	Coloration
Benzene-azo-resorcinol (Sudan G.)	Dull greenish yellow.
Benzene-azo-phenol	Yellow.
Amino-azo-ortho-toluene	Orange Yellow.
Amino-azo-benzene (Aniline Yellow)	Orange.
Dimethylamino-azo-benzene (Butter Yellow)	Red.
Benzene-azo-beta-naphthol (Sudan I)	Scarlet.
Xylene-azo-beta-naphthol (Sudan II)	Crimson.
Benzene-azo-alpha-naphthol	Crimson.
Benzene-azo-alpha-naphthylamine	Violet red.
Alpha-naphthalene-azo-beta-naphthol (Carminaph Garnet)	Violet.
Amino-azo-alpha-naphthalene	Blue.
Alpha-naphthalene-azo-alpha-naphthol (Sudan Brown)	Blue, fading,
Benzene-azo-benzene-azo-beta-naphthol (Sudan III)	Violet blue, fading.
Benzene-azo-beta-naphthylamine (Yellow A. B.) Ortho-toluene-azo-beta-naphthylamine (Yellow O. B.) Para-toluene-azo-beta-naphthylamine Meta-xylene-azo-beta-naphthylamine	Violet red, changing quickly to a clear yellow.
Bixin (Annatto) Carotin Xanthophyll	Blue, quickly fading.
Crocetin (Saffron)	Blue or green, fading
Curcumine (Turmeric)	Blue, becoming crimson in a few seconds.

The acetic anhydride solutions of Carotin also become blue when treated with dry iodine bromide, zinc chloride and many other reactive compounds. The colouring matter of orange peel even becomes blue or green when dissolved in glacial acetic acid and warmed.

Reactions with Boric and Concentrated Sulphuric Acids

Dimroth (Ber., 1910, **43**, 1391) and Formanek (*Untersuchungen der Farbstoffe auf spectroskopischen Wege*, 1908, p. 206) call attention to the observation of R. Schmidt that the solutions of the oxyanthroquinones in concentrated sulphuric acid show pronounced colour changes upon addition of boric acid, the latter forming boric esters. The red solution of carminic acid and the violet red solution of the closely related colouring matter of kermes become violet or blue when treated with a few crystals of boric acid and slightly warmed.

Spectroscopic and Spectrophotometric Methods

Formanek has developed a comprehensive scheme for the identification of colouring matters based on the measurement of the spectral position of maximum light absorptions shown by their dilute solutions in various solvents. The method is especially applicable with the xanthene dyes such as Rhodamine B and Eosine, since these give very sharply defined absorption bands. Other colouring matters showing rather characteristic absorption maxima are Methylene Blue, Naphthol Green B, and many of the anthroquinone derivatives. The bands shown by the azo dyes are in most cases rather broad or diffuse so that with a simple spectroscope it is very difficult to determine the exact position of the maxima. The treatises of Formanek and Grandmougin, and of Mullikin describe the absorption spectra of a large number of colouring matters. The spectroscope has been recommended also for the identification of Alkanet and the Chlorophylls. Photographs and descriptions of the rather complex absorption spectra of the chlorophylls and their derivatives are given by Willstaetter and Stoll (*Untersuchungen ueber das Chlorophyll*, Berlin, 1913).

By means of the spectrophotometer the exact magnitude of the light absorption of a dye solution can be determined in various parts of the spectrum and the absorption curve accurately plotted from these data. The curves determined for three or more solutions containing the same amount of a given dye but differing in their pH value (*i. e.* in N/10 sodium hydroxide, N/10 hydrochloric acid and N/10 acetate mixture) provide a very satisfactory description of its optical properties. Measurements taken at a few points with different solutions of suitably chosen hydrogen ion concentrations are quite useful for distinguishing isomeric or closely related dyes. See also Vol. VI.

DIRECT DYEING OF COTTON

The differentiation of the direct cotton dyes from the acid wool dyes by the use of cotton is described in Green's Systematic Survey of Organic Colouring Matters.

REACTIONS OF NATURAL COLOURING MATTERS

Relatively few good tests are known for the common natural colours. For properties useful in analysis, see especially the tables given by Loomis, *United States Department of Agriculture, Bureau of Chemistry Circular No.* 36. Some of the common properties con-

sidered most useful for the characterisation of different colours are summarised below.

By addition of concentrated hydrochloric acid, the yellow ethereal or alcoholic solutions of carotin and xanthophyll show little change, becoming perhaps slightly paler; green chlorophyll solutions become yellower or browner; annatto in ether or alcohol solution remains orange, not changing perceptibly with acid. Turmeric solutions in ether or alcohol show a pure yellow colour with more or less green fluorescence, and on addition of several volumes of concentrated hydrochloric acid the colour passes to orange red or carmine red. The orange or orange yellow solutions of logwood, also of the redwoods, barwood, sandalwood, camwood, and Brazil wood, become deep red with excess of hydrochloric acid. The slightly coloured neutral or faintly acid aqueous solutions of the flavonol colours of fustic, Persian berries, quercitron, etc., become intensely yellow with from 2 to 4 volumes of concentrated acid. Neutral or slightly acid solutions of cochineal, archil, saffron, and caramel show little change,

The slightly acid solutions of the various colouring matters show the behaviour described below, when treated with a little sodium hydroxide solution: carotin and xanthophyll, little change; chlorophyll, "brown phase" reaction; alkanet, deep blue; turmeric, orange brown; the redwoods, violet red; logwood, violet to violet blue. The flavonol colours become bright yellow; saffron remains yellow, showing little change. The red solutions of archil and the orange of cochineal become blue and violet, respectively. Caramel shows little change, becoming slightly deeper brown. The red fruit colours (in presence of air) become dull blue, green, or brown.

By sodium hydrosulphite in acid solution the yellow colouring matters are little affected. Logwood is almost decolorised, the colour returning imperfectly. Archil is decolorised, the colour returning when shaken with air. The reaction is more easily seen in alkaline solution. Cochineal shows no marked change. The anthocyanidins derived by hydrolysis from the red fruit colours are almost decolorised by hydrosulphite. Caramel is rendered slightly paler.

In the bromine test all colouring matters, except alkanet, are merely destroyed more or less completely by the halogen; hence they belong in general to Class E. The flavonol colours tend to become darker with the first addition of bromine. Alkanet (best in alcoholic solution) corresponds to Class B.

Ferric chloride gives no marked change with annatto, turmeric, or saffron, these perhaps, appearing somewhat browner. With the flavonol colours, colorations varying from dark olive green to black are produced. With the redwoods and logwood, very dark shades of violet, brown, or black are obtained. Cochineal becomes somewhat darker. Caramel is not affected. The solutions must be practically neutral.

By addition of alum solution the yellow color of logwood is changed to rose red (rather slowly). The redwoods are affected similarly. The pale yellow solutions of the flavonols become more strongly yellow, that of fustic developing a green fluorescence. Saffron and turmeric show little change.

Uranium acetate in neutral or nearly neutral solutions gives orange colorations with the flavonols. Turmeric becomes somewhat browner; saffron is not affected; cochineal becomes green; alkanet, yellowish green to bluish green; logwood, violet, quickly fading.

The coloration with concentrated sulphuric acid dropped on the dry colouring matter is for carotin and xanthophyll, blue, usually obtained with difficulty. Annatto and saffron also give blue colours; turmeric, a red; the flavonol colours, yellow or orange colorations; alkanet and archil give violet blue; logwood, red, changing to yellow.

The "brown phase" reaction[1] may be useful for the characterisation of chlorophyll, when this has not been previously treated with alkalies. The green ether or petroleum spirit solution of the colouring matter, when treated with a little methyl alcohol solution of potassium hydroxide, becomes brown, returning to green in a few moments.

The characteristic reaction of curcumin (turmeric) with boric acid may be conveniently carried out as follows: The aqueous or dilute alcoholic solution of the colour is treated with hydrochloric acid until the shade just begins to appear slightly orange. The mixture is then divided into two parts and some boric acid powder or crystals added to one part. A marked reddening quickly will be apparent, best seen by comparison with the portion to which the boric acid has not been added.

[1] Molisch, *Ber. bot. Ges.*, 1896, **14**, 16. Willstaetter and Stoll, *Untersuchungen über Chlorophyll.* Berlin, 1913, p. 144.

Quantitative Separation and Estimation of Food Colours

Although there are differences of opinion regarding the harmlessness of many of the coal tar dyes in use as food colours, governmental regulation has not yet been extended to the limitation of the amounts of the various permitted colouring matters allowed in food stuffs, it being held that excessive amounts of these substances would not be employed, as they would impair rather than improve the appearance of the coloured foods. Quantitative data are often useful, however, especially in cases where dye mixtures have been employed, as the use of objectionable grades of permitted food dyes is usually apparent from the relatively high percentages of subsidiary dyes in the mixtures. The better grades of food dyes do not contain subsidiary colouring matters in amounts above those shown below:

Naphthol Yellow S	0.03%	Martius Yellow
Orange 1	5%	Orange 2
Ponceau 3R	3%	Mono-sulphonated dyes
Tartrazine	3%	Mono-sulphonated dyes
Amaranth	4%	Di-sulphonated dyes
Indigo Carmine	5%	Mono-sulphonated dyes
Guinea Green B	3%	Lower-sulphonated dyes
Light Green S. F. Yellowish	5%	Lower-sulphonated dyes
Erythrosine	2%	Diodofluoresceine

After the preliminary qualitative examination has been made, if necessary, the methods described on pages 441–444 in many cases will serve to separate dyes almost completely from the accompanying food substances. Candies and beverages, two classes of products which must very frequently be examined for dyes, are readily treated with immiscible solvents, the appearance of the residue serving to indicate when all colouring matter has been extracted. With solid foods, consisting largely of nitrogenous substances, or containing considerable amounts of natural colouring matters as normal constituents, it is more difficult to make satisfactory separations from the food material.

The purified colouring matters obtained by any method will be in solution in dilute acid, amyl alcohol, or other solvent. Mixed dyes will often be separated from each other during the preliminary processes. The quantitative separation of mixtures is described in the following paragraphs, but when only two dyes are present these may be estimated in presence of each other by the spectrophotometer provided they are widely different in hue or show other well defined

differences in light absorptive properties. Unmixed dyes may, of course, be approximately estimated by ordinary colorimetric comparison also. Generally speaking, small amounts of dissolved colourless substances such as sugar, alcohol, sodium chloride, etc., do not greatly affect the accuracy of such estimations but care must be taken to keep the hydrogen ion concentrations of the solvents within definite limits. The regulation of the hydrogen ion concentrations is most easily accomplished by adding to the solutions such buffer mixtures as sodium acetate and acetic acid, primary and secondary phosphate, etc. (page 502).

Quantitative Separation of Mixed Colouring Matters

The amount of dye in a coloured food product will seldom exceed 0.01% and usually is considerably less, so that many of the common methods suitable for separating larger amounts of colouring matters are quite inapplicable. Chemical methods can occasionally be chosen which will give fair results, as for example, an adaption of the qualitative process already described for the isolation of Amaranth and Indigo Disulphoacid when present together. A general procedure that may be used with a great variety of colouring matters is the systematic fractionation between pairs of immiscible solvents (*J. Ind. Eng. Chem.*, 1913, **5**, 26; *U. S. Dept. Agr. Bur. Chem. Circular* 113; *J. Ind. Eng. Chem.*, 1920, **12**, 883; *J. Assoc. Off, Agr. Chem.*, 1921, **5**, No. 2, 122). This depends upon the selection of some pair of immiscible solvents in which the relative solubilities (or the distribution ratios) of the dyes to be separated are quite dissimilar. Such a pair of solvents can usually be found for a given binary dye mixture by reference to a table of relative solubilities such as that given on pages 464-476. More exact data for a single dye concentration are of no great advantage, as the distribution ratios change somewhat with variations in dye concentrations.

Portions, usually of thirty to fifty c.c., of the lighter solvent are measured into from two to four separatory funnels and the dye mixture in a measured amount of the heavier solvent is shaken successively with each portion of the lighter liquid. A second similar portion of the heavier solvent is then shaken out successively in the series of funnels, being passed through them in the same order as was the original solution. Two or more additional portions of the heavier solvent may be passed through the system in the same way.

If, for example, the relative volumes of the two solvents and the solubilities of the dyes are such that after the first shaking in the first funnel the lower layer contains 80% of one dye, 20% of the other, and these distribution ratios remain constant, then if four portions of each liquid are employed 98.3% of one dye and 1.7% of the other will be in the combined portions of the heavier solvent. In fractionating a mixture of two dyes in which one is present in relatively small proportion the conditions can be shifted rationally to give more accurate results, for example, by using a larger number of portions of the solvent in which the predominating dye is more soluble. Although the distribution ratios vary more or less with concentration and usually to a degree that makes such analytical processes unmanageable with colourless substances, the course of the separation is apparent to the eye in the case of dyes. The fractionation methods for this reason possess a reliability and flexibility with dyes that they quite lack with other compounds.

QUANTITATIVE SPECTRO COLORIMETRY[1]

The spectrophotometric method is particularly suitable for the precise estimation of the small amounts of colouring matters obtained in the analysis of foods. The basis of the procedure may be described as follows:

When a beam of monochromatic light passes through an absorbing medium the amount of light decreases in geometric ratio as the thickness of the layer increases in arithmetical ratio. In other words, every equally thick stratum of the medium absorbs the same fraction of the light entering it. If the absorbing medium is a dye dissolved in a colourless solvent the absorption varies more or less exactly with changes in the concentration in the same way that it does with changes in the thickness of the observed layer (Beer's law). This relation is expressed by the following equation in which t represents the fraction of the light unabsorbed after passing through unit thickness of the solution of unit concentration, T the fraction unabsorbed after passing through the same thickness of the concentration a.

$$t^a = T$$

Transforming this equation by taking the logarithms of both expressions and multiplying by -1 we obtain

[1] See also *Colorimetry* in Vol. VI.

$$-a \operatorname{Log} t = -\operatorname{Log} T$$

The negative logarithm of the fraction showing the unabsorbed light, or the negative logarithm of the transmission, is called the *transmissive index* or *extinction coefficient*, and the value for unit concentration and thickness of layer, the *specific transmissive index* or *specific extinction coefficient.*[1]

Various optical devices are in use for determining the light absorption constants of solutions under definite conditions. The author's experience indicates that a well constructed spectrophotometer of the Koenig-Martens form equipped with simple accessories (*J. Ind. Eng. Chem.*, 1920, **12**, 883) will give values agreeing closely with those obtained on the same sample by the exact methods used at the U. S. Bureau of Standards. (*Bur. Standards Bull.*, 448. See also Gibson, *Bur. Standards Scientific Paper* 349.)

The reading obtained on the spectrophotometer shows either the transmission, *i. e.*, the fraction of the light unabsorbed by the colouring matter in the solution, or some function of this value from which it may be calculated. For the purposes of chemical analysis it is most convenient to calculate the readings directly to the negative logarithms of the transmissions, since these values, the transmissive indices, are directly proportional to the corresponding concentrations, disregarding experimental errors and the variations due to lack of conformity with Beer's law. Deviations from Beer's law are not a serious source of inaccuracy, for they are usually small and within the working ranges of concentration and their values may easily be determined by measurements at several concentrations. To minimise experimental errors it is important that a correctly adjusted and aligned instrument of a good type be used and a solvent selected in which the colouring matter appears to exist more or less completely in a single molecular form. Water containing a definite small amount of acid, alkali, or buffer mixture to control the hydrogen ion concentration serves well for most of the sulphonated and basic dyes. Strong alcohol of accurately adjusted water content is convenient for dyes not readily soluble in aqueous liquids. Hydrogen ion regulators are not so necessary with alcoholic solvents except in cases in which strongly acid or alkaline solutions are desired. Distilled water alone

[1] Priest, *J. Optical Soc. Amer.*, 1920, 4, 186; Bunsen and Roscoe, Pogg., *Ann. Physik.* 1857; 101: 248, defined the extinction coefficient of an absorbing medium as the reciprocal of the thickness of the layer required to reduce the intensity of the incident light to 1/10 of its original value. They pointed out also that with a dissolved coloured substance this function could be taken as proportional to the concentration.

is satisfactory with many of the common acid dyes, but it is safer to add a buffer mixture to eliminate the variations due to dissolved carbon dioxide, alkali from the containers, etc.

The data by Gibson, McNicholas, Tyndall and Frehafer given below were obtained by using a solution one hundredth normal both to free acetic acid and to sodium acetate. Such a solution may be made by adding 20 c.c. of normal acetic acid and 10 c.c. of normal sodium hydroxide to a measured portion of the dye solution, then diluting to exactly 1000 c.c. The acetate mixture has been carefully studied by Walpole, *J. Chem. Soc.*, **105**, 1914, 2501, 2526. Other regulator mixtures are described by Sorensen. *Biochem. Zeitsch.*, 1901, **21**, 175 and *Ergebnisse Physiol.*, 1912, **12**, 437, 438; *Palitzsch. Biochem. Zeitsch.*, 1915, **70**, 341; Clark & Lubs, *J. Biol. Chem.*, 1916, **25**, 501.

Using solutions prepared by the author, Gibson, McNicholas, Tyndall and Frehafer (*Bureau of Standards Bull.* 440) have determined the optical constants of the seven dyes Naphthol Yellow S, Ponceau 3R, Orange 1, Amaranth, Erythrosine, Light Green S. F. Yellowish and Indigo Disulpho acid, their investigation being also a careful comparative study of the methods of spectrophotometry. The following values, taken from the report, show the specific transmissive indices or specific extinction coefficients as calculated for solutions containing one centigram of the pure dye in 1000 c.c. These values have been chosen with special consideration to the need of the analytical chemist, and their cologarithms are also given to facilitate calculation.

Corresponding values for tartrazine are approximately $k_{435.8} = 0.525$, $k_{546.1} = 0.0001$. A specimen of Guinea Green B of good commercial grade gave the following specific transmissive indices for the pure dye in 0.01 normal acetate mixture as solvent;

$$k_{435} = 0.27, \quad k_{546} = 0.16, \quad k_{579} = 0.595, \quad k_{623} = 1.36$$

The proportion of pure colouring matter in the product was estimated by titration with decinormal titanium trichloride, one c.c. of the standard solution being considered to correspond to 0.03453 grm. dye.

The specific transmission indices of four oil-soluble dyes in light of wave length 435.8mμ are as follows: alcohol of specific gravity 0.8055 (93% by weight) being employed as solvent. Butter Yellow, 0.93; Yellow A. B., 0.56; Yellow O. B., 9.54; Sudan 1, 0.43 (*J. Ind. Eng.*

TABLE 7 —SPECIFIC TRANSMISSIVE INDICES

Name of dye[a]		Specific transmissive index (k) at wave lengths (mμ)								
		Hg 435.8	He 447.2	He 471.3	He 501.6	Hg 546.1	Hg 576.9	Hg 579.1	He 587.6	He 667.8
Naphthol yellow S	k	0.475	0.40$_5$	0.150	0.0106	0.00013	*0.00002	*0.00002	*0.00001	*0.00001
	colog	.323	.393	.824	1.975	3.886	4.7	4 7	5.	5.
Orange I	k	.47	.64	.88	.65	.110	.021	.019	.013	*.001$_5$
	colog	.328	.194	.055	.187	.958	1.678	1.721	1.886	2.82
Ponceau 3 R	k	.137	.171	.32	.51	.29$_5$	*.032	*.027	*.014	.0001$_5$
	colog	.863	.767	.495	.292	.530	1.495	1.568	1.854	3.824
Amaranth	k	.109	.126	.22	.38$_5$	.34$_5$	.130	.115	.065	.0008$_5$
	colog	.962	.900	.657	.414	.462	.886	.939	1.187	3.07
Erythrosine	k	.027$_5$	.040	.126	.42$_5$	.264	.0075	.0058	*.0019	*.00001
	colog	1.561	1.398	.900	.372	.578	2.125	2.237	2.721	5.
Indigo disulpho acid	k	.035	.036$_5$	.036	.046	.157	.31	.32	.36	.062
	colog	1.456	1.438	1.444	1.337	.804	.509	.495	.444	1.208
Light green S F yellowish	k	.204	.132	.024	.016	.100	.33	.35	.44	.32
	colog	.690	.879	1.620	1.796	1.000	.481	.456	.356	.495

[a] Acetate mixture was used as a solvent for all but Erythrosine, for which water was used.

Chem., 1920, **12**, 883). Schertz (*Agr. Research*, 1923, **26**, 383) has studied the quantitative determination of carotin, using carefully recrystallised colouring matter of melting point 174° C. In ether, at concentrations ranging from 0.070 to 0.400 centigrams per litre, the specific transmissive index at 435.8mμ was found to be about 1.99. Determinations made under the same conditions, except with petroleum spirit and with 95% alcohol as solvents, gave in both cases values of from 1.912 to 1.919.

It may be seen from the figures given above that the commoner colouring matters are most conveniently estimated with the spectrophotometer when the solutions are of concentrations ranging from 5 to 0.5 centigrams per litre, so that in analytical practice at least, the absorption constants are best expressed in terms of these units. Holmes (*J. Amer. Chem. Soc.*, 1924, **46**, 208) has given spectrophotometric data for the sulphonated indigotins.

For the estimation of the concentration of a solution containing only a single fairly pure dye it is almost always best to determine the transmissive index in light for which the absorption is relatively high (*i. e.*, at or near the maximum of an absorption band), as the absorption due to small amounts of any coloured impurities present affect a high value proportionally less than they do a lower one. When two or more dyes are to be estimated in the same solution it is necessary to have as many measurements as there are dyes present, and these should be made in spectral regions in which the relative absorptions are widely different. A white light source, such as a Mazda lamp, may be used with almost any spectrophotometer, but the values found, while reproducible with the same apparatus, are affected by errors not easily corrected, so that with the simpler instruments at least, monochromatic light is usually more satisfactory. The Mercury Arc is the only commercial device now obtainable for producing radiation which can be used with suitable ray filters to give powerful monochromatic light. This light source gives strong radiation of wave lengths 435.8mμ, 546.1mμ, 786.9mμ, and 579.1mμ, which serve fairly well for examining most dye mixtures. For the measurements in red light necessary with solutions containing green and blue dyes the spectral apparatus may be set on the rather faint red mercury line at 623.mμ (visible when a Quartz Mercury Arc Lamp is used) and the mercury lamp then replaced by a Mazda light source.

If the solution containing a single dye is examined in a layer 1 cm. in thickness the concentration is equal to the observed transmissive index divided by the specific transmissive index of the dye as found with the same solvent, radiation, etc.

When two dyes are present in a solution their concentrations may be calculated from the following equations in which

X = Concentration Dye A
Y = Concentration of Dye B
T_m = Transmissive index observed with radiation m
T_n = Transmissive index observed with radiation n
a_m = Specific transmissive index of the dye A with radiation m
a_n = Specific transmissive index of the dye A with radiation n
b_m = Specific transmissive index of the dye B with radiation m
b_n = Specific transmissive index of the dye B with radiation n

$$T_m = a_m X + b_m Y$$
$$T_n = a_n X + b_n Y$$

The relation between the corresponding variables for three dyes present together are shown by equations that are similar in form, and in cases in which the colouring matters are widely different in absorptive properties as for example, the brown mixtures made from Tartrazine, Amaranth and Light Green S. F. Yellowish, fairly satisfactory analyses may be calculated from measurements in three properly chosen regions of the spectrum. The results are usually quite inaccurate, however, since the effect of the experimental error becomes very pronounced. The method is convenient and accurate for the routine analysis of binary dye mixtures and may be combined advantageously with a fractionation procedure when three or more colouring matters are present.

The equations just given may be expressed in somewhat more convenient form when the numerical values of the specific transmissive indices (given on page 503) are used. Thus, if a solution containing Naphthol Yellow S and Ponceau 3R shows the transmissive indices T_{436} and T_{546} at 436.mμ and 546.mμ respectively, and if N represents the concentration (in centigrams per liter) of the Naphthol Yellow S, and P that of the Ponceau 3R, then

$$N = 2.10T_{436} - 0.98T_{546}$$
$$P = 3.39T_{546} - 0.001T_{436}$$

The analysis of mixtures, especially of those containing three or more dyes may be facilitated occasionally by making measurements in more than one kind of solvent or at different hydrogen ion concentrations. It is necessary, of course, to know the specific transmissive indices of all the dyes in the mixture for each of the solvents used, and a set of purified or analysed dyes should be at hand so that comparative data under any given conditions can be obtained readily.

The spectrophotometer provides an excellent means for establishing the identity of colouring matters. The transmissive indices at several points should be in the same ratio as the corresponding specific transmissive indices of the known dye supposed to be identical to the one under examination. The changes in equilibrium for a given change in the pH value of the solvent may be followed accurately spectrophometrically giving very conclusive results in some cases.

ANALYSIS OF COMMERCIAL FOOD COLOURING MATERIALS

COAL TAR DYES AND DYE MIXTURES

The examination of commercial dyestuffs is described in Vol. VI. Explicit methods for the analysis of dyes permitted in the U. S. A. are described by Seeker (Hesse, *U. S. Dept. Agr. Bur. Chem. Bull.* 147). With some modifications and additions by the author these are embodied in the Tentative Methods of the A. O. A. C. as adopted November 1920. (*J. Assoc. Off. Agr. Chemists*, 1921, **5**, No. 2, 196–218). The procedure outlined for the estimation of moisture, salt, sodium sulphate, insoluble matter, and arsenic in Orange 1 may be applied also for the estimation of these impurities in Guinea Green B. Lower sulphonated subsidiary dyes in the latter may be separated by a systematic fractionation by means of amyl alcohol and gasoline mixture and a dilute aqueous solution of equimolecular amounts of acetic acid and sodium acetate.

A preliminary qualitative examination of the colouring matter of commercial food dyes and dye mixtures may be made as outlined on page 439. The method described by Price (*U. S. Dept. Agr. Bur. Animal Ind. Circ.* 180) for the qualitative separation of the seven dyes: Naphthol Yellow S, Ponceau 3 R, Orange 1, Tartrazine, Light Green S. F. Yellow, Amaranth, Erythrosine and Indigo Disulpho

acid has been modified by Estes (*J. Ind. End. Chem.*, 1918, **8**, 1123) and by Ingersol (*J. Ind. Eng. Chem.*, 1917, **9**, 955) to include Tartrazine and otherwise to extend its applicability.

Natural Colouring Matters

The analysis of food colouring preparations made from so-called natural or vegetable dye stuffs should include tests for poisonous heavy metals, especially arsenic and tin, and the estimation of the colouring matters. Materials that owe their colouring power mainly to a single chemical substance may be examined with the spectrophotometer, a sample of the pure colouring matter being carried through in the same way to determine its specific absorptive constants when these are not known. Few data have been published[1] showing specific transmissive indices of the commoner natural colouring matters, perhaps mainly because of the uncertainty in correcting observed spectrophotometric readings for errors due to width of slit, scattering light, etc. Such corrections are, of course, unnecessary when the values are to be used only for comparison with others taken under the same conditions. Fairly pure specimens of several of the natural colouring matters may be bought on the market or made in the laboratory without great difficulty. The author has used with success the preparative methods given by Zwick (*Ber.*, **30**, 1972) for bixin; Liebermann, Hamburger, (*Ber.*, **12**, 1179) for quercitron; Perkin (*Chem. Soc. Trans.*, 1904, **85**, 63) for curcumine; and Schunck and Marchlewski (*Ber.*, **27**, 2981) for carminic acid, obtaining well crystallised products.

Carminamide may be prepared by digesting crystallised carminic acid with four parts of strong ammonia water in a sealed tube for four or five weeks at room temperature. The mixture is evaporated to dryness over sulphuric acid *in vacuo*, the dark amorphous mass of the amide thus obtained being further purified by dissolving it in glacial acetic acid, filtering and evaporating the filtrate until most of the solvent is removed. The pasty mixture thus obtained is filtered on a Witt plate, the carminamide washed with little glacial acetic acid, then with dry ether, and finally dried over sulphuric acid. The tinc-

[1] In two recent papers (*J. Agr. Research*, 1923, **26**, 383 and 1925, **30**, 253, Schertz has given transmissive indices for the widely distributed yellow colouring matters carrotin and xanthophyll, and has discussed the conditions necessary for their exact analytical estimation. When ether solutions were used, and concentrations expressed in centigrams per litre, the values found for the specific transmissive indices were 1.99, and 2.09 for carotin and xanthophyll, respectively.

torial value of such materials as commercial caramels, saffrons, etc. may be expressed by comparison with standard products or by transmissive indices observed under specific conditions. (*J. Assoc. Agr. Chem.*, 1916, **2**, 2, 164.)

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BENZENE AND ITS HOMOLOGUES

BY J. BENNETT HILL, PH. D.

The hydrocarbons of the benzene groups have the general formula C_nH_{2n-6} and are therefore richer in carbon than the other common series of hydrocarbons. They are characterised by the presence of the benzene ring, indicated conventionally by the benzene hexagon which without additional groups represents benzene, C_6H_6, the simplest member of the series. The higher members have one or more of the hydrogens at the corners of the hexagon replaced by methyl or higher alkyl groups.

Benzene was discovered by Faraday in 1825 in the condensed liquid from illuminating gas. It was subsequently prepared by Mitscherlich in 1834 by the distillation of calcium benzoate, and was shown by A. W. Hoffman in 1845 to be present in coal tar.

The predominant commercial source of benzene and its homologues is at present the products from the distillation of coal and especially from coke oven operation. Coal tar, so produced, contains benzene and its homologues and is worked up for these products. The larger portion, however, of the lower benzene hydrocarbons in coke oven operation goes on with the gas and is scrubbed out by a heavy absorbent oil from which it is recovered as coke oven light oil. Illuminating gas, whether coal gas, water gas or oil gas, contains benzene hydrocarbons which are sometimes recovered by scrubbing the gas. Where no scrubbing is done some of these products condense out in the gas holders and mains as drip oil or holder oil which, when worked up, constitutes another source of benzene and its homologues.

Benzene is produced under certain conditions from the cracking of petroleum at high temperatures. This process is not, up to the present time, a commercially important source of the pure hydrocarbons.

Benzene and its homologues are produced from the various crude tars or distillates containing them by a refining process. This consists in preparing suitable fractions by distillation, washing these

with sulphuric acid to remove the unsaturated and readily polymerisable impurities, washing with a solution of sodium hydroxide, and fractionally distilling to produce the desired products.

PROPERTIES OF BENZENE HYDROCARBONS

Some of the physical properties of benzene and its important homologues are shown in the following table:

	Empirical formula	B. p.	M. p.	Sp. gr. at 15.5° C.	Ref. ind. at 15° C.
Benzene	C_6H_6	80.4	5.48	.8845	1.5038
Toluene	C_7H_8	110.5	−94.5	.8710	1.4992
p-Xylene	C_8H_{10}	138.4	16	.8661	1.4985
m-Xylene	C_8H_{10}	139.5	−54	.8691	1.4996
o-Xylene	C_8H_{10}	144.3	−28	.8851	1.5082
Mesitylene	C_9H_{12}	164.5	−57	.8585	....
Pseudocumene	C_9H_{12}	169.4	....	.8747	......
Hemimellithene	C_9H_{12}	175	−15	.901	.....
Cymene	$C_{10}H_{14}$	177	......	.8569	......

In general, the benzene hydrocarbons are distinguished physically from the paraffin hydrocarbons by a higher sp. gr., a higher ref. ind. and a more powerful and aromatic odour. They possess better solvent properties towards most substances. Their vapours are more toxic.

Chemically the benzene hydrocarbons are much more reactive than the paraffins. Most of the reactions given for benzene on page 512 are typical of the series. They react with sulphuric acid to form sulphonic acids, with nitric acid to form nitro-compounds, and with chlorine to form substitution and addition products.

The benzene hydrocarbons may be reduced with the addition of hydrogen to the hexahydro-aromatic compounds or naphthenes, having the general formula C_nH_{2n}, isomeric with the olefines, but having a ring structure. The naphthenes behave in many respects like saturated hydrocarbons, being incapable of forming additive compounds, offering great resistance to the action of oxidising agents, and, when yielding, splitting up completely, with formation of carbon dioxide and water as the chief products. They are not acted on in the cold by bromine, fuming sulphuric acid, or nitric acid of sp. gr. 1.4; but fuming nitric acid, or a mixture of nitric and sulphuric acids, converts them into the nitro-derivatives of the benzene hydrocarbons. Naphthenes have been found largely in Caucasian petroleum, and

are present also in American petroleum, especially from the California and Gulf Coast areas.

Benzene, C_6H_6

Nearly pure benzene may be obtained from coal distillation products by the usual refining process and careful fractionation. The commercially pure article so obtained may be further purified by partially crystallising and separating the mother liquor from the crystals, this operation being carried out one or more times.

Pure benzene is a colourless, very limpid, highly refractive liquid of a peculiar and rather agreeable odour. It has a specific gravity of 0.8845 at 15.5°, and a coefficient of expansion at this temperature of 0.00128 per degree. It freezes at 5.48° to a mass of white needles and boils without decomposition at 80.4°. Its vapour is highly inflammable and burns with a very smoky flame.

Flame propagation in an explosive mixture of benzene vapour and air is slow compared with that in mixtures of the vapours of the paraffin hydrocarbons with air. For this reason benzene has come into great importance as a fuel for internal combustion engines in the automobile industry, since, even in mixtures with gasoline, the benzene has the effect of checking flame propagation and decreasing the tendency to detonate or "knock."

Benzene is practically insoluble in, though communicating its odour to, water, but is miscible in all proportions with methyl, ethyl, and amyl alcohols, ether, chloroform, carbon tetrachloride, petroleum spirit, turpentine, absolute phenol, and fixed and volatile oils.

Hot benzene dissolves sulphur, phosphorus, and iodine. It is an excellent solvent for gutta-percha and india-rubber, which are left unaltered on evaporation. It also dissolves waxes, fats, and fatty acids. It is interesting to note that it is not a good solvent for paraffin wax.

Benzene may be heated to 400° in a sealed tube without change; but when passed through a tube heated to a bright redness it yields hydrogen, together with diphenyl, $C_{12}H_{10}$, and other hydrocarbons. Benzene is not acted on by distilling it with metallic sodium. Alkali hydroxides have no effect on it. It dissolves entirely when heated to 100° for some hours with 4 or 5 times its volume of concentrated sulphuric acid. The resulting liquid contains benzenesulphonic acid, $C_6H_5SO_3H$, and is colourless if pure benzene be employed.

At very high temperatures, or when fuming sulphuric acid is employed, benzenedisulphonic acids are produced. Under the influence of oxidising agents benzene yields a number of interesting products, according to the treatment to which it is subjected. Thus:

(*a*) By the action of air on benzene vapour in the presence of a catalyst, such as vanadium pentoxide, and under the influence of heat, maleic acid is formed.

(*b*) By the action of manganese dioxide and concentrated sulphuric acid, benzene yields carbon dioxide, formic acid, and water, together with small quantities of benzoic, phthalic, and terephthalic acids.

(*c*) By the action of concentrated nitric acid, benzene is readily converted into nitrobenzene, $C_6H_5NO_2$; and by the continued action of the acid, especially if hot or mixed with sulphuric acid, dinitrobenzenes $C_6H_4(NO_2)_2$, are produced.

By the action of chlorine or bromine in the dark or in diffused light, benzene is converted into chlorinated or brominated derivatives, in some cases 5 out of the 6 atoms of hydrogen being replaced. In direct sunlight, chlorine and bromine form additive compounds with benzene, of which benzene hexachloride, $C_6H_6Cl_6$, is a type. Iodine alone has no action on benzene, but when a mixture of benzene with iodine and iodic acid is heated iodobenzenes are formed.

By prolonged treatment with hydriodic acid, under high pressure, benzene is converted into benzene hexahydride or cyclohexane, C_6H_{12}, a substance isomeric with hexene.

Separation and Recognition of Benzene.—When in a pure state and in appreciable quantity, benzene is readily recognisable by its smell, sp. gr., and b. p. The chemical tests capable of ready application are few, the most satisfactory being the formation of nitrobenzene with nitric acid, followed by the action of reducing agents on the nitro-compound, and recognition of the aniline so formed.

This test is only applicable to benzene in approximate purity, or at least free from certain admixtures. When present in appreciable proportions in complex mixtures the following procedure is suggested for its separation from interfering substances and its identification:

The liquid is first treated with a 10% solution of sodium hydroxide to remove phenols and other acid substances. The aqueous layer is drawn off, and the oil layer subjected to distillation in a flask provided with a fractionating column. The fraction between 65° and 100° contains most of the benzene and is cut separately. This

fraction is treated cold with about 6% by volume of concentrated sulphuric acid, separated from the acid layer and washed with dilute sodium hydroxide solution. It is then carefully fractionated with an efficient fractionating column, and the fraction between 78° and 83° collected separately. The benzene is contained in this fraction, but is probably diluted with hydrocarbons of other series of nearly the same boiling point. The fraction may be treated with a mixture of twice its weight of concentrated nitric acid and three times its weight of concentrated sulphuric acid. The benzene fraction is added to the mixed acids with constant shaking at a temperature of about 50–60°. The mixture is poured slowly into water, the temperature being kept moderately low. The oil layer[1] is separated from the aqueous layer, and a portion allowed to evaporate. Nitrobenzene may be readily recognised by its odour and indicates the presence of benzene in the original mixture. The nitrobenzene may also be reduced with zinc and hydrochloric acid and the resultant aniline detected with bleaching powder.

According to Iw. Trifonow (*Z. anorg. allgem. Chem.*, 1922, **124**, 136) benzene may be detected in admixture with toluene, xylene and aliphatic hydrocarbons by means of its reaction with pernitric acid. 5 c.c. of the unknown liquid are treated with 5 to 10 c.c. of a mixture of equal volumes of 3% hydrogen peroxide and 4% sodium nitrite solution. The mixture is emulsified by shaking, and 2 to 3 c.c. of 2N H_2SO_4 added, and the whole shaken. After standing a few minutes the hydrocarbon layer is poured off and a small piece of solid NaOH added. If benzene was present, a red ring is formed around the solid, or, if present in large amounts, the whole liquid is coloured red.

Estimation of Benzene.—For the estimation of benzene in admixture with other hydrocarbons, particularly petroleum hydrocarbons, the Atlantic Refining Company has adapted the general method of C. E. Fawsilt (*J. Chem. Soc.*, 1919, **115**, 801). The method consists in determining the average molecular weight of the mixture by the lowering of the freezing point of acetic acid and then determining the lowering of the freezing point of pure benzene by the mixture. Since the benzene content of the mixture does not affect this freezing point, the per cent. of benzene may be calculated as follows:

[1] If both benzene and paraffins are present in considerable quantity, two oil layers may form, the upper being a solution of nitrobenzene in hydrocarbons and the lower a solution of hydrocarbons in nitrobenzene.

$100x$ = weight per cent. benzene in mixture
M = average molecular weight of mixture
Δ = lowering of freezing point of
W = grm. of benzene by
w = grm. of the unknown mixture
k = molecular freezing point lowering of benzene per grm. = 5120
M_1 = molecular weight of benzene = 78
M_2 = average mol. wt. of other compounds

$$\frac{1}{M} = \frac{x}{M_1} + \frac{1 - x}{M_2} \qquad (1)$$

$$M_2 = k\frac{(1 - x)w}{\Delta(W + xw)} \qquad (2)$$

Combining (1) and (2)

$$100x = \frac{100M_1}{Mw} \cdot \frac{kw - M\Delta W}{M_1\Delta + k}$$

in which the various values may be substituted giving the per cent. by weight of benzene in the mixture. It should be noted that this method gives actual benzene and is not influenced by other aromatic hydrocarbons.

COMMERCIAL BENZENE

The pure benzene of commerce, as obtained by the refining of coal distillation products, is required to conform to strict specifications. The following is taken from a Barrett Company specification as typical for the United States:

Colour.—Not darker than $K_2Cr_2O_7$ solution of 0.3 mg./100 c.c.
Distillation.—Start to dry within 1°
Acid Wash.—Not darker than No. 2.
Sp. Gr. @15.5°.—0.8820 – 0.8860
Freezing Point.—Not less than 4.75°

The distillation, specific gravity, and sulphuric acid wash tests are described in a later section under Commercial Benzols (see p. 523). Tests are also frequently made for freezing point, thiophene content (see page 516) and carbon disulphide.

Freezing point is determined by cooling the liquid in a test tube placed inside a larger tube to serve as an air bath, and this tube immersed in ice-water. The benzene is stirred with an accurate

thermometer graduated in tenths of a degree. When crystallisation begins the temperature rises due to previous supercooling, reaching a maximum and then falling again. This maximum temperature is taken as the freezing point (or melting point).

It is pointed out by W. J. Jones (*J. Soc. Dyers and Col.*, 1919, **35**, 45–47) that the purity of the benzene may be estimated from an accurate freezing-point determination by assuming that all the impurities exert the same lowering effect as toluene, and applying the relation

$$\% \text{ purity} = 90.51 + 1.73\, t$$

in which t is the observed freezing point of the sample.

Carbon disulphide is generally estimated by precipitation as copper xanthate. The following is the detailed method as used by the Barrett Company (*J. Ind. Eng. Chem.*, 1918, **10**, 1009):

The reagents used consist of a solution of alcoholic potassium hydroxide prepared by dissolving 110 grm. of potassium hydroxide in 900 grm. of absolute alcohol, a standard solution of copper sulphate (1 c.c. equivalent to 0.0075 grm. CS_2), prepared by dissolving 12.475 grm. of $CuSO_4.5H_2O$ in one litre of distilled water, a potassium ferrocyanide solution, and an acetic acid solution.

Exactly 50 grm. of the benzol to be tested shall be weighed into an Erlenmeyer flask, mixed well with 50 grm. of alcoholic potassium hydroxide solution, the flask stoppered and the mixture allowed to stand for 5 or 6 hrs. at the ordinary temperature. The carbon disulphide by this treatment is converted to potassium xanthate. The mixture shall then be shaken up with about 100 c.c. of water, and the aqueous layer separated from the benzol. This washing shall be repeated several times with 30 c.c. portions of water, adding the washings to the original water solution. The solution shall then be diluted to 250 c.c., and an aliquot portion removed, neutralised with acetic acid, and titrated with copper sulphate solution. The end-point shall be determined by placing a drop of the solution on a filter paper next to a drop of potassium ferrocyanide solution. The completion of the titration is indicated by a reddish brown zone of copper ferrocyanide. The per cent. CS_2 is calculated from the relation,

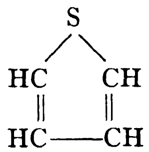
$$\frac{\text{c.c. } CuSO_4 \text{ soln.} \times 3.75}{\text{c.c. taken for titration}} = \% CS_2.$$

The above quantity of alcoholic potassium hydroxide is sufficient if the sample contains less than 5% of carbon disulphide. If it contains more, a smaller sample should be taken. In this case, the formula for calculation must be modified accordingly.

THIOPHENE, C_4H_4S

This compound, while not structurally a member of the benzene group, is nevertheless so closely related in occurrence and in chemical and physical properties that it is considered at this point.

Thiophene has the formula,



It occurs in coal tar and, at least partly, comes through the refining process with the benzene. Commercially pure benzene normally contains about 0.05% of this compound.

Thiophene is a colourless liquid, boiling at 84°, having a specific gravity of 1.07 at 15°, and an odour closely resembling that of benzene. Its sulphur is tightly bound in the ring and it exhibits none of the properties of the alkyl sulphides. Thus it is not attacked by the alkali metals. Treated with sulphuric aid it sulphonates more readily than benzene. Nitric acid oxidises it rapidly, although the mono- and dinitrothiophene have been prepared. Bromine forms monobromthiophene, boiling at 150° and having a density of 1.652 at 23°; or, if a larger quantity of bromine is used, forms dibromthiophene, $C_4H_2Br_2S$, which is a colourless liquid boiling at 211° and of 2.147 sp. gr. at 23°. In its reactions this body closely resembles thiophene.

Thiophene gives characteristic colour reactions with many reagents. The most notable of these is the deep blue indophenin produced on agitating thiophene with isatin and strong sulphuric aicd. When the proportion of thiophene is large, a dirty brown is produced. If the mixture be warmed, the same reaction is produced by many of the derivatives of thiophene, including dibromthiophene, thiophenesulphonic acid, $C_2H_2S.SO_2H$, and thiophenic acid, $C_2H_2S.COOH$. According to H. Kreis (*Chem. Zeit.*, **26**, 523, 1902), if

benzene, containing even very minute amounts of thiophene, is shaken with a very dilute solution of thalline (*p*-methoxytetrahydroquinoline) and a little (1.42) nitric acid, the acid becomes coloured intensely violet. This reaction is delicate with a solution of thalline and thiophene in benzene as dilute as 1–100,000. The violet coloration is not permanent; it changes gradually to reddish and finally to yellow.

Thiophene is normally removed from benzene by repeated treatment with sulphuric acid or by crystallisation of the benzene. Other chemical means are frequently used. For example, Haller and Michel (*Bull. Soc. Chim.*, 1896, 1065) employ aluminium chloride. 1% is sufficient when the sample contains but little thiophene; but when the proportion is greater, 4 or 5% may be needed. The aluminium chloride is agitated with the benzene and then allowed to stand, when a viscous, reddish product collects at the bottom. The benzene may be distilled off at once or after separation from this liquid.

Estimation of Thiophene in Benzene.—The volumetric method of Denigès (*Bull. Soc. Chim.*, 1896, 1065) makes use of the precipitation of the thiophene as a complex mercury compound. 2 c.c. of the sample are placed in a 60 c.c. stoppered flask with 30 c.c. of methyl alcohol, free from acetone, and 10 c.c. of a mercuric sulphate solution prepared by dissolving 50 grm. red mercuric oxide in 200 c.c. of sulphuric acid and diluting to a litre. The flask is closed and the mixture allowed to stand for about 20 minutes. An insoluble compound, $2HgO.HgSO_4.C_4H_4S$, is formed, and is removed by filtration. 21 c.c. of the filtrate (1 c.c. of the sample) are placed in a litre flask with 350 c.c. of water, 15 c.c. of ammonium hydroxide solution, 10 c.c. N/10 potassium cyanide solution, and 5 or 6 drops of a 20% solution of potassium iodide, and the whole well shaken. If not perfectly clear, a gentle heat may be applied, and then, after cooling, N/10 silver nitrate is added until a permanent turbidity results. The amount of thiophene (x) can be calculated by the formula:

$$x = 2.8(n - 0.3);$$

in which n equals the number of c.c. of silver nitrate solution used.

The colorimetric method of Schwalbe, as modified by the Barrett Company, is as follows: The reagents required are a solution of isatin in C. P. sulphuric acid, made by dissolving 0.1 grm. of isatin in 1000

c.c. of acid, pure thiophene, and thiophene-free benzene. A 0.05% stock solution of thiophene in the thiophene-free benzene is accurately prepared, and from this six solutions containing, respectively, 0.005, 0.004, 0.003, 0.002, 0.001 and 0.0005% thiophene are made up as required.

Unless the material to be tested is known to contain less than 0.005% of thiophene, two dilutions of the sample are made—one by diluting 10 c.c. to 100 c.c. with thiophene-free benzol, the other by diluting 1 c.c. to 100 c.c. with thiophene-free benzol.

Ten c.c. of the undiluted sample are measured into a 1 oz. French square, glass-stoppered bottle, and 10 c.c. of isatin solution are added. The bottle is well shaken and allowed to stand for one hour. At the same time this test is made similar tests are made with the two diluted samples and with the six standard solutions. At the end of the hour the colorations of the acid layers of the diluted and undiluted samples are compared with the colorations of the acid layers from the standard solutions.

If the undiluted sample agrees with one of the standards, the per cent. of thiophene in the sample is that of the standard agreeing with it. If one of the diluted samples corresponds with one of the standards, the per cent. is obtained by multiplying by 10 if it is the 10% solution, and by 100 if it is the 1% solution that agrees with the standard.

TOLUENE

Methylbenzene, $C_6H_5CH_3$

Toluene, like benzene, is generally prepared by the refining of coal tar naphthas. It may also be prepared by various synthetic reactions and is a product resulting from the dry distillation of tolu-balsam. It closely resembles benzene in its properties but may be readily distinguished from it by the following characteristics:

1. The odour is quite distinct from that of benzene.
2. The b. p., 110.5°, is considerably higher than benzene.
3. The m. p., −94.5°, is very much lower than benzene which freezes at 5.48°.

Pure toluene has a specific gravity of .871 at 15.5° and a coefficient of expansion of 0.00114 per degree. It is important on account of being the basis of numerous dyes and synthetic chemicals. Its trinitro derivative is the important explosive TNT.

By the action of concentrated nitric acid, toluene is converted into one or more nitrotoluenes, or dinitrotoluenes; but when boiled with dilute nitric acid it is oxidised with formation of benzoic acid and other products. When treated with excess of hot concentrated sulphuric acid, toluene forms two isomeric toluenesulphonic acids, $C_7H_7HSO_3$.

The pure toluene of commerce is generally required to distil completely within 1° and to meet strict specifications on sulphuric acid wash and on sp. gr. The methods of making these tests are described under Commercial Benzols (page 522).

XYLENES

Dimethylbenzenes, $C_6H_4(CH_3)_2$

Xylene occurs in three isomeric forms, as shown on page 520. All three isomers occur in coal tar naphthas, as well as the isomeric compound ethyl benzene. Meta-xylene is present in much the largest amount, with the para- next, and the ortho- considerably less.

The xylenes are obtained from the refining of coal tar naphthas, and the separate isomers may be prepared from this source in a fair degree of purity by very careful fractional distillation. Meta-xylene is the most important commercially of the three. The mixtures of the xylenes are of commercial importance as commercial xylol and "solvent naphtha" (see page 522).

The table on page 520 shows some of the principal points of difference in the physical and chemical properties of the xylenes.

Chromic Acid Mixture.— A solution of 8 grm. of potassium dichromate in a mixture of 10 grm. of sulphuric acid and 90 c.c. of water.

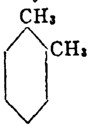
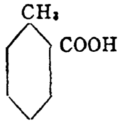
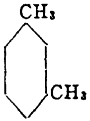
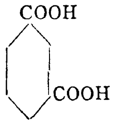
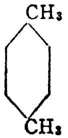
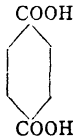
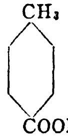
Diluted Nitric Acid.—Nitric acid (sp. gr. 1.4), diluted with twice its volume of water.

Nitric-sulphuric Acid Mixture.—One part of fuming nitric acid (sp. gr. 1.48) and two parts of concentrated sulphuric acid are mixed and allowed to cool.

The first two reagents are applied by boiling the hydrocarbon with excess of each under an inverted condenser for several hours.

The nitric-sulphuric acid mixture is used by adding to about 3 c.c. of it one drop of the hydrocarbon and boiling gently in a test-tube. The mixture is poured into water, and the nitro-derivatives filtered out and recrystallised from boiling alcohol.

PROPERTIES OF XYLENES

Name	Solidifying point, °	B. p., °	Sp. gr., 15.5°	(For preparation of reagents, see page 519) Results of action of			
				Diluted sulphuric acid	Chromic acid mixture	Diluted nitric acid	Mixture of nitric and sulphuric acids
Ortho-xylene 1-2 dimethylbenzene 	−28	144.3	.8851	Easily soluble	Entirely decomposed	Orthotoluic acid 	Liquid products
Meta-xylene 1-3 dimethylbenzene 	−54	139.5	.8691	Soluble	With difficulty, Isophthalic acid 	Slight action	Trinitrometaxylene, $(C_6H(NO_2)_3(CH_3)_2$ m. p. 181°
Para-xylene 1-4 dimethylbenzene 	16	138.4	.8661	Insoluble	Terephthalic acid 	Paratoluic acid 	Trinitroparaxylene, $C_6H(NO_2)_3(CH_3)_2$ m. p. 139°

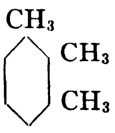
The pure xylenes of commerce are generally specified by their distillation ranges, wash tests, specific gravities, and sometimes paraffin contents. The common distillation ranges are 3° and 5°, the upper and lower limits being specified. The testing methods are described under Commercial Benzols (page 523).

The following method for the analysis of commercial xylene is due to J. M. Crafts (*Compt. rend.*, 1892, **114**, 1110.)

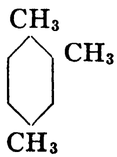
A weighed amount of the sample (10 to 20 grm.) is poured on to 2.5 times its weight of concentrated sulphuric acid in a tube of hard glass. The depth of the xylene layer in mm. is noted, the tube is sealed and heated to 120°, with occasional violet shaking. The tube is allowed to cool, opened, 3 or 4 volumes of a mixture of equal parts of hydrochloric acid and water added, the mixture shaken well and allowed to stand for an hour at room temperature. An insoluble layer of saturated hydrocarbons is formed. This is measured and separated in a separatory funnel, the solvent acid returned to the tube, which is resealed and heated to 122° for 20 hours. By this treatment 97% of the 1-3-xylene forms a layer, which can be measured, and after separation distilled and weighed. A small amount of impurity is removed by distillation. The 1-2- and 1-4-xylenesulphonic acids undergo but little decomposition at 122°, but at 175° the hydrocarbons are regenerated. If these be dissolved in 3 parts of concentrated sulphuric acid, the solution cooled, and 1 part of concentrated hydrochloric acid added, 1-4-xylenesulphonic acid is thrown down in crystals, which should be collected on an asbestos filter, washed with concentrated hydrochloric acid until the washings no longer react with barium chloride, and the precipitate allowed to dry in the air to constant weight. The crystals have the composition $(C_8H_9SO_3H)_2 + 3H_2O$; the weight multiplied by 0.4977 gives the xylene.

TRIMETHYLBENZENES, $C_6H_3(CH_3)_3$

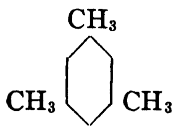
There are three isomeric trimethylbenzenes as shown below, all occurring in coal tar naphthas.



hemimellithene



pseudocumene



mesitylene

Their physical properties are shown in the table on page 510. Pseudocumene occurs in the largest quantity.

Isomeric with the trimethyl benzenes are three methyl-ethylbenzenes, propyl benzene and isopropyl benzene (cumene.) These various hydrocarbons of the empirical formula C_9H_{12} make up the principal part of the commercial coal tar solvent known as "hi-flash naphtha."

CYMENE, $C_6H_4(CH_3)(C_3H_7)$. PARA-METHYL-ISOPROPYL BENZENE

This compound has been shown to be present in coal tar naphthas. Its principal source, however, is the product known as "spruce turpentine," which is a by-product in the manufacture of spruce pulp by the sulphite process. It boils at 177° and has a refractive index of 1.4905 at 12.5°.

COMMERCIAL BENZOLS

In addition to the commercially pure hydrocarbons which have already been described, there are available as commercial products a large number of refined mixtures of the hydrocarbons in various proportions, generally specified by their distillation ranges. These are known as benzols, toluols, xylols, etc., or generically as benzols.

A typical line of commercially pure hydrocarbons and benzols are marketed in the United States by the Barrett Company under specifications as contained in part in the following table:

	Acid wash	Sp. gr. 15.5°	Distillation
Nitration benzene	2	.8820–.8860	Complete within 1°
Commercially pure benzol	4	.875 –.886	Complete within 2°
90% Benzol	6	.875 –.887	90–95% @ 100°; dry at 120°
Motor benzol	12	....	Start @ 76–82°; 60% @ 100°; 90% @ 120°; dry @ 170°.
Nitration toluene	2	.8690–.8730	Complete within 1°
3° Xylol	6	.865 –.870	Complete within 3° between 137.2°–140.5°
5° Xylol	6	.860 –.870	Complete within 5° including 139.5°
Solvent naphtha	12	....	Not over 5% @ 130°; 90–95% @ 160°; dry @ 180°
Hi-flash naphtha	10 (60° acid)	.865 –.890	Between 150°–200°

It should be pointed out that the terms 100% benzol, 90% benzol, 50% benzol, etc., frequently used, do not refer to the actual per cent. benzene, but only to the amount distilled off at 100° in the distillation test described below.

Until recent years there were marketed a series of crude benzols which were not acid treated, were far from "water white" and were therefore unrefined. Among these products were Straw Colour Benzol, Straw Colour Toluol, etc. Since the demand for motor benzol has become so great, these products have practically disappeared.

Methods of Test

The methods given below are those generally accepted in the United States. Many of them are taken from the published methods of the Barrett Company (*J. Ind. Eng. Chem.*, 1918, **10**, 1006).

Sp. gr. is generally determined by the Mohr-Westphal balance at 15.5° or at 25°. Where greater accuracy is required a capped weighing bottle may be employed.

Distillation.—The assembly of the apparatus is shown in Fig. 7.

The distillation flask shall be a 200 c.c. side-neck distilling flask having the following dimensions:

	Mm.
Diameter of bulb	73
Outside diameter of neck	24
Inside diameter of neck	21
Length of neck	105
Inside diameter of side tube	5
Length of side tube	127
Side tube joined to neck above the base of the neck	52

The allowable variation from the above dimensions shall be not more than 5%. (See Fig. 7.)

The thermometer shall conform to the following specifications (T. S. T. M. High-Distillation Thermometer).

Type.—Etched stem, glass.

Liquid.—Mercury.

Range and Subdivision.—0 to 400° in 1°.

Total Length.—378 to 384 mm.

Stem.—Plain front, enamel back, suitable thermometer tubing. Diameter, 6.0 to 7.0 mm.

Bulb.—Corning normal or equally suitable thermometric glass.

Length, 10 to 15 mm.

Diameter, 5.0 to 6.0 mm.

Distance to 0° Line from Bottom of Bulb.—25 to 35 mm.

Distance to 400° Line from Top of Thermometer.—30 to 45 mm.

Filling above Mercury.—Nitrogen gas.

Top Finish.—Glass ring.

Graduation.—All lines, figures and letters clear cut and distinct. The first and each succeeding 5° line to be longer than the remaining lines. Graduations to be numbered at each multiple of 10°.

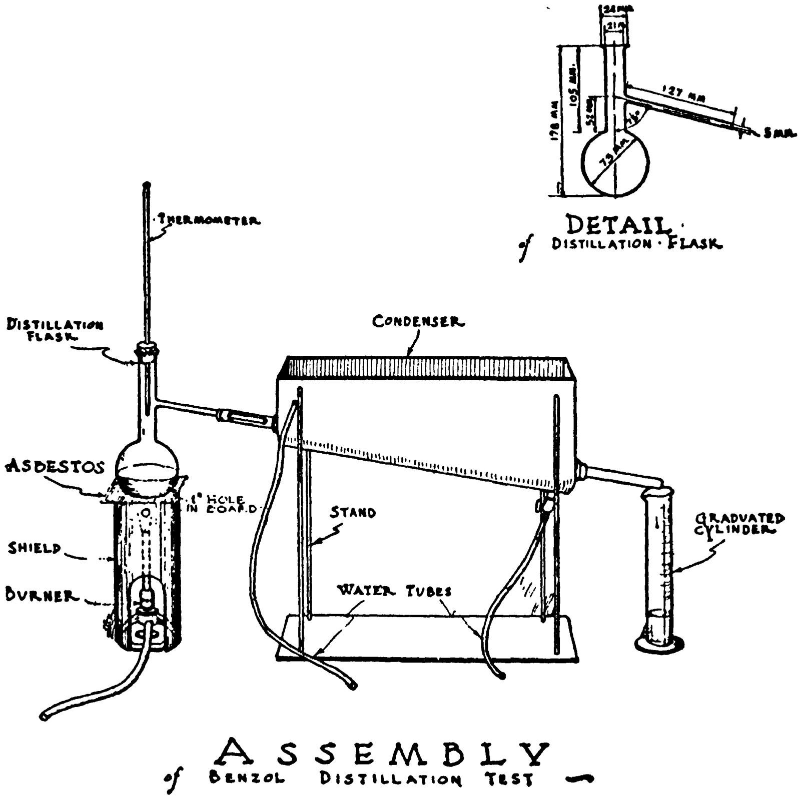


FIG. 7.

Immersion.—Total.

Special Marking.—"A. S. T. M. High Distillation," a serial number, and the manufacturer's name or trade mark shall be etched on the stem.

Scale Error.—The error at any point of the scale up to 370° when the thermometer is standardised as provided below shall not exceed 1°.

Standardisation.—The thermometer shall be standardised immersed in the testing bath to the top of the mercury column, at the ice point and at temperature intervals of approximately 50° up to 370°.

Test for Permanency of Range.—After being subjected to a temperature between 360 to 370° for 24 hours, the accuracy shall be within the limit specified.

Case.—The thermometer shall be supplied in a suitable case on which shall appear the marking: "A. S. T. M. High Distillation, 0 to 400°."

Note.—For the purpose of interpreting these specifications the following definitions apply:

The total length is the over-all length of the finished instrument.

The diameter is that measured with a ring gauge.

The length of the bulb is the distance from the bottom of the bulb to the beginning of the enamel backing.

The top of the thermometer is the top of the finished instrument.

For pure benzene and toluene the thermometer shall have a range of 70–120° in 0.2° and for xylene 110–160° in 0.2°. Both thermometers shall conform to the following dimensions:

	Mm.
Total length	Not over 305
Bulb length	Not over 20
70° mark to bottom of bulb	80 to 100
Graduation per inch	Not over 35
Stem diameter	5 to 7 mm.
Bulb diameter	5 to 7 mm.

The distillate shall be condensed in a straight tube of ½ in. internal diameter and 24 in. in length, set at an inclination of 75° to the vertical. At least 15 in. of tube shall be cooled with cold water in a trough condenser.

The heating flame shall be derived from a Bunsen burner, and the entire flame shall be blue.

An ordinary 100 c.c. cylinder, graduated at intervals of 1 c.c. shall be used for the receiver. Graduations must be clear cut and distinct. The graduate shall be approximately 1 in. in diameter. The mark for each 10 c.c. shall be longer than the intermediate markings and shall be plainly numbered.

The flask shall be supported on a sheet of ⅛ in. asbestos board, 6 in. × 6 in., with a hole in the centre 1 in. in diameter for distillations substantially complete below 140° and 1½ in. for higher boiling

liquids. The asbestos board shall be supported on a circular metal shield enclosing the Bunsen flame. The flask shall be so placed that the vapour tube will extend at least 2 in. into the condenser tube.

The thermometer shall be held in the neck of the distillation flask by means of a cork stopper in such a position that the top of the bulb shall be opposite the lower side of the side tube and central in the neck of the flask.

The sample to be tested shall be poured into a 100 c.c. graduated cylinder and 100 c.c. of the material shall be carefully measured and transferred to the distilling flask. The flask shall be put in connection with the condenser and the thermometer introduced through a tightly fitting cork. The graduated cylinder which was used to measure the oil shall not be rinsed out, but shall be placed under the lower end of the condenser tube to catch the distillate. The flask shall be heated up slowly, especially after ebullition has begun, so as to allow the mercury column of the thermometer to become fully expanded before the first drop distils off.

The flame shall then be turned up and the distillation conducted at the rate of 5 c.c. per min. (2 drops per sec.). This rate must be exact. The distillation shall be continued to dryness. The total yield of distillate shall not be less than 95 per cent.

A temperature reading shall be taken when the first drop of distillate falls into the receiving cylinder. Additional temperature readings shall be taken when 5%, 10%, each 10% thereafter, and 95 % of distillate have distilled over. A final reading shall be taken of the "dry" point, which is the point at which liquid just disappears from the bottom of the flask.

Care must be taken to quickly remove the burner as the last bubble is evaporated, otherwise, too high a dry point may be produced by superheating.

Temperature readings may be corrected for barometer by *adding* to the observed temperature the factor given below, multiplied by the number of mm. the barometer stands *below* 760 mm.

Benzene	0.043°
Toluene	0.047°
Xylene	0.050°

Where true temperature readings are required, it is necessary to correct the observed readings for the emergent stem of the thermometer. This correction may be calculated as follows:

$$C = 0.00016\,N(T - t)$$

where C is the correction to be *added* to the reading

T is the temperature reading

t is the average temperature of the exposed stem

N is the number of degrees exposed which is equal to the difference in degrees between the temperature reading and the point on the scale of the thermometer about 1 cm. below the bottom of the cork.

Sulphuric Acid Wash.—This test is a determination of the colour imparted to sulphuric acid by the material, and is a measure of the carbonisable impurities contained in the sample and therefore of its degree of refinement. A low wash test indicates a well-refined product.

The set of colour standards with which wash tests shall be compared shall consist of fifteen bottles (French square flint glass, glass stoppered, one ounce capacity) each containing one of the coloured solutions made up as given below, the bottle being sealed.

For making up the standards the following basic solutions shall be used:

A—59.4965 g. $CoCl_2.6H_2O$ (nickel-free) made up to 1000 c.c. with a mixture of 25 c.c. 31% HCl and 975 c.c. H_2O.

B—45.054 g. $FeCl_3.6H_2O$ made up to 1000 c.c. with a mixture of 25 c.c. 31% HCl and 975 c.c. H_2O.

C—3.5 volumes of Solution A + 36.5 volumes Solution B + 90 vol. of H_2O.

D—3.5 volumes of Solution A + 36.5 volumes of Solution B. (No water is added.)

E—Solution of K_2CrO_4 saturated at 21° C.

F—One volume of a solution of $K_2Cr_2O_7$ saturated at 21° C. + one volume of H_2O.

As standard colour solutions to be used for comparison the following shall be made up and numbered from 0 to 14:

No. 0—Pure water.

No. 1—One volume of Solution C + 1 volume of H_2O.

No. 2—5½ volumes of Solution C + 2 volumes of H_2O.

No. 3—Solution C as such.

No. 4—One volume of Solution D + one volume of H_2O.

No. 5—5½ volumes of Solution D + two volumes of H_2O.

No. 6—Solution D as such.

No. 7—5 volumes of Solution E + 2 volumes of water.
No. 8—Solution E as such.
No. 9—7 volumes of Solution E + ½ volume of Solution F.
No. 10—6½ volumes of Solution E + 1 volume of Solution F.
No. 11—5½ volumes of Solution E + 2 volumes of Solution F.
No. 12—One volume of Solution E + 1 volume of Solution F.
No. 13—Two volumes of Solution E + 5 volumes of Solution F.
No. 14—Solution F as such.

These standard solutions shall, in all cases, remain stoppered and sealed to prevent evaporation.

The test bottles shall be one-ounce, French square, glass-stoppered flint glass bottles identical in every respect with those containing the standard solutions.

Seven c.c. of 96 ± 0.5% C. P. sulphuric acid shall first be placed in a test bottle and approximately 21 c.c. of the material to be tested shall be added. The bottle after being stoppered shall be thoroughly and vigorously shaken for 15 to 20 sec. and allowed to stand for the specified time.

The resulting colour of the acid layer shall be compared with the set of standards, and the number of the bottle in the standard set corresponding to the test bottle shall be noted.

If the colour of the acid layer is not uniform, it should be carefully mixed by slowly inverting the bottle once or twice.

For solvent naphtha the test is allowed to stand only five minutes before reading. Hi-flash naphtha with 96% acid is generally too dark for the standards. Tests may be made on such materials by substituting 60° acid. In this case the fact should be noted in the result.

Sulphur.—Sulphur is best estimated by burning a weighed sample in a bomb with oxygen in the presence of a small amount of a solution of sodium hydroxide. The contents of the bomb are well washed out with water, the sulphur, present largely as sulphite, oxidised to sulphate by bromine water, the solution acidified with hydrochloric acid, and the sulphur precipitated and weighed as barium sulphate.

The method recommended by the British Engineering Standards Association (Benzol for Motor Fuel No. 135–1921) is as follows:

The apparatus used is shown in Fig. 8, and consists of a U-tube *C* loosely packed with cotton wool and suspended in the beaker *B*,

which is partly filled with water. One arm of the U-tube is connected with one limb of Y-tube *D*, the second limb of which is connected with the wash-bottle *A*.

The third limb of the Y-tube leads into the tube *E* of clear silica, 15 inches long by ½ inch internal diameter, which tube is almost completely packed with small pieces of platinised quartz.[1] The outlet tube of *E* is connected with wash-bottles *F* and *G*, containing sodium hypobromite solution. A vacuum service is connected with *G* so that a current of air may be drawn through the apparatus.

To perform the analysis the silica tube is heated to redness for a length of about 4 inches.

The taps of *C* are opened and the vacuum adjusted to give a steady stream of air through the apparatus. The tap on *C* nearer the silica

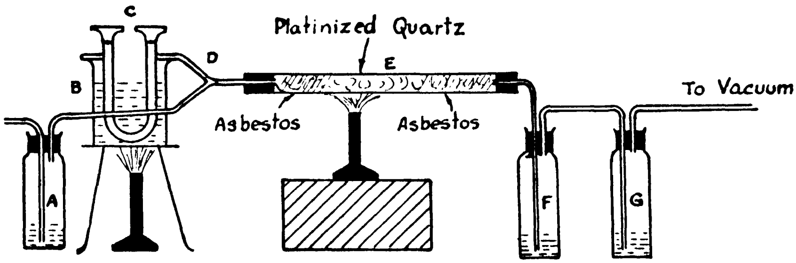


FIG. 8.

tube is then closed, and that further from the tube removed, and 2 c.c. of the benzol under examination rapidly run into *C* from a standardised pipette. The top is then quickly replaced in the open position, and the tap nearer the silica tube opened slightly. As soon as benzol vapour begins to be drawn through by the vacuum the platinised quartz begins to glow brightly. The air passing through *A* is adjusted until the reaction is steady. The combustion is allowed to go on until the glow on the platinised quartz dies down with the taps on *C* full open. The water in the beaker is then heated to boiling, and the combustion proceeded with until all the benzol has been oxidised. In a well-conducted experiment the total time taken for this operation should be not less than twenty, and not more than forty-five minutes.

The contents of *F* and *G* are then washed into a beaker, acidified with HCl, heated to boiling until all bromine is expelled, and slight

[1] The quartz consists of small pieces of semi-transparent partially devitrified quartz platinised by immersion in a 5 % solution of platinum chloride, pouring off the excess of solution, drying and igniting. Platinised asbestos must not be used.

excess of barium chloride added. The contents are allowed to stand over night, filtered, and the $BaSO_4$ ignited and weighed.

Then:

Percentage of sulphur in the sample =

$$\frac{(\text{weight of } BaSO_4) \times (0.1377)}{(\text{specific gravity of sample}) \times (2)}.$$

The sodium hypobromite is prepared by shaking 8 c.c. of bromine[1] with 1 litre of 10 per cent. sodium hydroxide solution, 100 c.c. of solution being used for each analysis. As no commercial caustic soda or bromine is free from sulphur compounds, a "blank" must be performed for each preparation of solution.

Paraffins.—The estimation given below shows any unsolphonatable hydrocarbons as paraffins.

The test vessel consists of a Babcock milk bottle. The graduated portion of the neck contains 2 c.c., divided into 10 major divisions. 10 c.c. of the benzol to be tested shall be measured into the Babcock bottle, and 10 c.c. of fuming sulphuric acid containing 20% free SO_3 slowly added to it, cooling the bottle in a bath of ice water during the addition of the acid, and shaking the bottle vigorously after each addition in order to mix the contents thoroughly. After the addition of the acid is complete, the bottle shall be removed from the ice bath, shaken until the temperature rises to about 40°, and then alternately cooled and shaken for a period of 15 min., keeping the temperature below 40°. Then the mixture shall be cooled again, 10 c.c. more of the fuming sulphuric acid added, the whole mixed thoroughly and shaken and cooled as above, keeping the temperature below 40°. Finally the mixture shall be allowed to stand for 30 min. at a temperature of about 35°. Then the bottle shall be cooled in ice water, and water added through the capillary steam of a funnel so that it enters below the surface of the acid. The water shall be added in small portions very cautiously, and the bottle thoroughly shaken and cooled after the add'tion of each portion. When sufficient water has been added to bring the level of the liquid well up on the graduated portion of the neck, the bottle shall be placed in a centrifuge and whirled for 5 min.

The paraffins present will rise to the surface, and their volume shall then be read off in terms of the graduations on the neck of the bottle.

[1] The bromine used must be as pure as possible. Some of the bromine obtainable on the market is heavily contaminated with sulphur compounds.

This reading (in major divisions), multiplied by 2, gives directly the volume % of paraffins in the original material.

On diluting the sulphonation mixture with water it will frequently happen that a small quantity of solid sulphone will be formed, which, on centrifuging, will form a layer between the paraffins and the acid layer. This sulphone should not be mistaken for paraffins.

Colour.—Colour of commercial benzols is generally compared with standard solutions of potassium dichromate in 1% H_2SO_4 contained in regulation 4 oz. oil sample bottles.

The colour may also be determined by means of the Saybolt Chromometer. This instrument consists of two vertical tubes, one of which is empty and contains a slot for the insertion of a standard coloured glass. The second tube is filled with the liquid to be tested, which is then drawn down through a stop cock near the bottom, to such a depth that when viewed through the eyepiece the colour intensity matches the standard glass. The height of the liquid in the tube is converted to the arbitrary Saybolt scale by a chart attached to the instrument.

Saybolt Chromometer readings may be interpreted in terms of mg. $K_2Cr_2O_7$ per 100 c.c. 1% H_2SO_4 by the following table:

Saybolt colour	Mg. potassium dichromate / 100 c.c. of 1% sulphuric acid solution
25	0.20
24	0.30
23	0.37
22	0.45
21	0.55
20	0.65
19	0.75
18	0.85
17	0.95
16	1.10
15	1.25
14	1.35
13	1.50
12	1.65
11	1.75
10	1.85
9	1.95
8	2.05
7	2.17
6	2.30
5	2.40
4	2.55
3	2.65
2	2.75
1	2.85
0	3.00

COAL TAR LIGHT OILS

It is frequently necessary to evaluate for its content of benzene, toluene and xylene a crude light oil obtained from one of the various sources of such oils.

To accomplish this the light oil is roughly fractionated with a Hempel column up to a vapour temperature of 170°. The fraction so obtained is washed in a separatory funnel with 1% by volume of 60° Bé. sulphuric acid. The sludge is left to settle and drawn off, and the oil treated with three successive portions of 2% by volume each of 66° Bé. sulphuric acid, drawing off the sludge after treatment. The oil should be kept cool during all the acid treatments. The oil is washed with dilute sodium hydroxide solutions to neutralise and is then steam distilled until oil ceases to come over. The refined oil is separated from the water and submitted to very careful fractional distillation through a highly efficient column. The distillation should be so conducted that both the benzene and toluene fractions come off at substantially constant temperature and that the intermediates between the benzene and toluene and between the toluene and xylene are very small compared with the size of the charge. The benzene, toluene, and xylene cuts are measured and referred back to the original light oil.

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ANILINE AND ITS ALLIES

BY A. B. DAVIS

Aniline, $C_6H_5.NH_2$

Aniline exists in minute quantity in coal tar, but is ordinarily produced by nitrating benzene and reducing the resulting nitrobenzene, $C_6H_5NO_2$, by suitable means.

If the treatment with nitric acid be carried further, dinitrobenzene is produced, and this by reduction is converted into phenylene-diamines or diamino-benzenes, $C_6H_4(NH_2)_2$.

If the reduction of nitrobenzene be effected by alkaline reagents, 2 molecules unite, and azobenzene, $C_6H_5.N:N.C_6H_5$, is produced. On further treatment of this (especially in alcoholic solution) it is converted into hydrazobenzene, $C_6H_5.NH.NH.C_6H_5$, which by intramolecular change is transformed into benzidine or di-para-amino-diphenyl, $NH_2.C_6H_4.C_6H_4.NH_2$, by the action of mineral acids. The relationship of aniline to the allied bases is shown below:

Aniline (aminobenzene)	Aniline	Aniline (phenylamine)
$NH_2.C_6H_4.H$	$C_6H_5.NH.H$	$C_6H_5.NH.H$
Phenylene-diamine	Phenylhydrazine	Diphenylamine
$NH_2.C_6H_4.NH_2$	$C_6H_5.NH.NH_2$	$C_6H_5.NH.C_6H_5$
Benzidine	Hydrazobenzene[1]	Hydrazobenzene[1]
$NH_2.C_6H_4.C_6H_4.NH_2$	$C_6H_5.NH.NH.C_6H_5$	$C_6H_5.NH.NH.C_6H_5$

[1] Hydrazobenzene does not have basic properties.

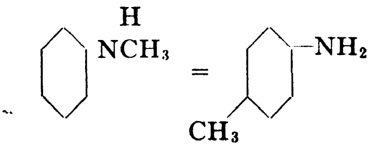
Aniline forms two classes of homologues. The true homologues (Class A) co-exist with aniline in coal tar, and are derived from aniline by the substitution of one or more methyl groups for a corresponding number of the hydrogen atoms of the benzene nucleus. They are ordinarily obtained by nitrating the corresponding hydrocarbons prepared from coal-tar naphtha, and reducing the resulting nitro-derivatives. Thus:

Hydrocarbon	Nitro-derivative	Amino-derivative
Benzene— $C_6H_5.H$	Nitrobenzene— $C_6H_5.NO_2$	Aniline— $C_6H_5.NH_2$
Toluene— $C_6H_4(CH_3).H$	Nitrotoluene— $C_6H_4(CH_3).NO_2$	Toluidine— $C_6H_4(CH_3).NH_2$
Xylene— $C_6H_3(CH_3)_2.H$	Nitroxylene— $C_6H_3(CH_3)_2.NO_2$	Xylidine— $C_6H_3(CH_3)_2.NH_2$
Cumene— $C_6H_2(CH_3)_3.H$	Nitrocumene— $C_6H_2(CH_3)_3.NO_2$	Cumidine— $C_6H_2(CH_3)_3.NH_2$

Isomeric modifications are known of all the members of the series except those in the first line.

The pseudo-homologues of aniline (Class B) are derived from aniline by the replacement of one or both of the hydrogen atoms of the amino-group by methyl or other alkyl radical. Similar substitutions can be effected in the amino-groups of toluidine, xylidine, etc.

These alkyl substituted anilines (Class B) are obtained by the action of methyl chloride or other alkyl salt on aniline, or of the corresponding alcohol on the hydrochloride or other salt of aniline. *p*-Toluidine has also been obtained in a very interesting manner by heating the hydrochloride of methyl-aniline[1] to 350° in a sealed tube, when, change of position of the atoms within the molecule takes place, thus:



By the same process methyl-toluidine may be converted into xylidine, and this by consecutive steps into a pseudo-cumidine, isoduridine and amino-pentamethylbenzene. By treating aniline hydrochloride with aniline, diphenylamine or phenylaniline, $C_6H_5.NHC_6H_5$, is obtained.[2]

Substitution of the hydrogen atoms of aniline and its homologues can also be effected by acid groups both in the benzene nucleus and in the amino group. In the latter case the derivatives are

[1] If the hydriodide of methyl-aniline be similarly treated, ortho- or meta- toluidine is obtained.

[2] Diphenylamine and aniline hydrochloride cannot be caused to react with formation of triphenylamine, $(C_6H_5)_3N$; but this substance can be obtained by the action of mono-brom-benzene on di-potassium aniline:

$$C_6H_5Br + C_6H_5.NK_2 = (C_6H_5)_3N + 2KBr.$$

called anilides and are quite different from the substances resulting from the substitution of chlorine for the hydrogen of the benzene nucleus. In the substances of the latter class the basic character is either much weakened or entirely destroyed. Most of the derivatives exist in several isomeric modifications, according to the position of the substituting radicals in the benzene nucleus. Examples of the substances of this class are:

Aniline-sulphonic acid or sulphanilic acid, $C_6H_4(SO_3H).NH_2$.

Nitraniline, $C_6H_4(NO_2).NH_2$.

Bromaniline, $C_6H_2Br.NH_2$.

Trichloraniline, $C_6H_2Cl_3.NH_2$.

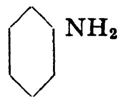
Mixed substitution products, belonging at once to two or more of the foregoing classes, are obtained by suitable means. As examples may be mentioned:

Paranitracetanilide,	$C_6H_4(NO_2).NH(C_2H_3O)$
Paranitroso-dimethylaniline,	$C_6H_4(NO).N(CH_3)_2$

By the action of nitrous acid on a cold solution of a salt of aniline a salt of diazobenzene is obtained. This and the allied products obtained by similar means from the homologues and analogues of aniline form the starting-point of the numerous and important colouring matters known as the "azo-dyes."

By the action of reducing agents on the salts of diazobenzene, phenylhydrazine, $C_6H_5NH(NH_2)$, is obtained. This substance has already been fully described.

Aniline. Amino-benzene. Phenylamine.

$C_6H_7N = C_6H_5.NH_2 =$ 

Aniline was first obtained in 1826 by Unverdorben by the dry distillation of indigo, and received the name *crystalline*. Runge in 1834 obtained it from coal tar, and termed it *kyanol*. The name *aniline* is due to Fritsche, who in 1841 obtained it by distilling indigo with caustic alkali. The name *benzidam* was given it in 1842 by Zinin, who prepared it by reducing nitrobenzene by hydrogen sulphide. The name *phenamine* has also been proposed for it. Aniline was first accurately described in 1843 by A. W. Hofmann.

Aniline occurs to a very limited extent ready-formed in the products of the distillation of coal, bone, and peat. Almost the whole

of it, however, is obtained indirectly from coal-tar benzene and the action of reducing agents on the resulting nitrobenzene ("Aniline Oils.") Aniline may also be obtained by passing ammonia and benzene vapour through a red-hot tube; $C_6H_6 + NH_3 = H_2 + C_6H_7N$. It is also formed together with diphenylamine by the reaction of phenol and ammonia. The best yield is obtained by heating phenol to about 330° for 20 hours with ammonium chloride and magnesia or oxide of zinc (or ammonio-zinc chloride, $Zn(NH_3)_2Cl_2$). Aniline is also obtained by numerous other reactions.

Aniline may be purified by fractional distillation and conversion into the acetyl derivative. This is recrystallised from water, and on saponification yields pure aniline.

Pure aniline is a colourless, oily liquid, of faintly vinous odour and aromatic, burning taste. It refracts light strongly, but has no rotatory action. Aniline, when very pure, freezes at 6°, but a slight impurity greatly reduces its solidifying point. It boils at 184.4°, and distils unchanged.

The sp. gr. of aniline is 1.0389 at 0° and 1.0342 at 4°, compared with water at 4°; and 1.0254 at 15°, compared with water at the same temperature. The coefficient of expansion is 0.000818.

Aniline becomes yellow or brown on exposure to air and light, especially at elevated temperatures, a resinous substance being ultimately formed. The change is due to oxidation, and does not occur *in vacuo* or in the dark. According to A. Bidet (*Compt. Rend.*, 1889, **108**, 520), aniline and toluidine prepared by the reduction of pure nitro-derivatives are colourless after distillation, and though they become yellowish in a few days, light has no further effect on them, and even this change Bidet attributes to the presence of aminothiophene, $C_4H_3S.NH_2$.

Aniline is only slightly soluble in water, requiring 28.7 parts at the ordinary temperature, but being more soluble in hot water. Water also dissolves in aniline, 5 parts being taken up by 100 of aniline at the ordinary temperature, and somewhat more at higher temperatures. The greater part can be separated by distillation, the water passing over first, but the last traces can only be removed by prolonged digestion over alkali hydroxides.

Aniline is soluble in all proportions in a 50% aqueous solution of its hydrochloride, and in smaller proportions in more dilute solutions.

Aniline dissolves readily in ethyl and methyl alcohols, ether, acetone, chloroform, carbon disulphide, and volatile hydrocarbons.

Aniline is itself a solvent for sulphur, phosphorus, indigotin, camphor and colophony, but does not dissolve caoutchouc or copal. It is employed sometimes as a solvent for aniline blue.

Aniline is a powerful poison, coagulating albumin and producing symptoms similar to those caused by nitrobenzene.

According to Letheby and Turnbull the action of aniline is chiefly on the nervous system. According to Grandhomme, the first symptom in slight cases of poisoning by aniline, caused by inhaling the vapour, is a blue colour on the edge of the lips, while the gait becomes unsteady, the speech thick, the head affected, and the face pale, and the appetite fails completely. Alcohol aggravates the symptoms. In more severe cases, such as may arise from the saturation of the clothes with aniline, the lips become dark blue or black, and the vertigo is so violent that standing becomes impossible. According to Wöhler and Frerichs, aniline does not exert any poisonous action on dogs. Runge found the aqueous solution to kill leeches and the parts of plants immersed in it.

Certain manufacturing companies avoid poisoning cases by careful regulations concerning the diet and clothes of workmen. The eating of food in the plant manufacturing amino and nitro compounds is prohibited.[1]

Aniline has marked basic properties, a long series of well-defined and crystallisable salts being obtained from it. It has, however, no action on phenolphthalein, litmus or turmeric, though it affects a few of the more delicate vegetable colours, such as haematoxylin. It expels ammonia from its salts at a boiling temperature, but is itself displaced in the cold. Aniline decomposes the solutions of many metallic salts, with precipitation of the corresponding hydroxides. When heated with strong sulphuric acid, aniline is converted into *p*-amino-benzene-sulphonic acid (sulphanilic acid). With hot fuming sulphuric acid, di-sulphonic acids are produced.

In presence of an excess of acid, aniline imparts a deep yellow colour to pine-wood and alder-pith.

According to Friswell, on adding cupric sulphate to an aqueous solution of aniline an apple-green crystalline precipitate is formed; or in extremely diluted solutions a green coloration.

[1] Auer, E. G.

Cold aqueous solutions of aniline salts are converted by treatment with nitrous acid (or a nitrite and mineral acid) into salts of diazobenzene. On boiling the solution, phenol is formed, with evolution of nitrogen.

If aniline, or one of its salts in the solid state, be treated with a drop of chloroform, and then solid potassium hydroxide or a strong solution of potassium hydroxide in alcohol be added, and the whole gently heated by immersing the tube in hot water, a peculiar and highly unpleasant odour will be produced, due to the formation of phenyl-carbamine, $C_6N_5.NC$. The reaction, which is known as "Hofmann's isonitrile test," is produced by other aromatic monamines, and by acetanilide.

Under the influence of oxidising agents aniline gives products and reactions which vary considerably according to the oxidising agent employed, thus:

(*a*) When aniline is treated with excess of nitric acid, and the mixture evaporated at 100°, the base is decomposed, with formation of a brown substance. With smaller proportions of nitric acid various coloured products are formed, including picric acid.

(*b*) When treated with dilute sulphuric acid and manganese dioxide, aniline yields ammonia and quinone, $C_6H_4O_2$, but the greater part of the product undergoes still further change.

(*c*) If aniline is dissolved in strong sulphuric acid, and a few drops of a solution of potassium dichromate are added, a red colour is produced, which rapidly changes to deep blue.

(*d*) On treating aniline, or one of its salts in a solid state, with strong sulphuric acid, and then adding a minute fragment of manganese dioxide or other oxidising agent (in the manner described under "strychnine,") a fine purple coloration is produced. A better result is obtainable by employing electrolytic oxygen; in this form the test is the most delicate and satisfactory which can be applied.

(*e*) Chlorine acts on dry aniline with great violence, producing a black mass containing trichloraniline, $C_6H_2Cl_3NH_2$. Bromine behaves similarly; and, on adding bromine-water to the aqueous solution of an aniline salt, a precipitate of tribromaniline is formed. On the other hand, Mills and Muter (*J. Soc. Chem. Ind.*, 1885, **4**, 96) state that aniline in solution in carbon disulphide reacts with bromine, probably forming an additive compound.

(*f*) When a solution of aniline is treated with a dilute solution of bleaching powder, avoiding excess, a fine purple coloration results, which gradually changes to brown. When carefully applied, the reaction is delicate and characteristic. The colour is destroyed by ether.

(*g*) If a minute quantity of aniline be treated with an aqueous solution of phenol, and a solution of bleaching powder be then gradually added, the reagent produces yellow striæ, which change to a greenish-blue. The test, which is due to Jacquemin, is said to be very delicate.

The following work on the reduction of aniline has been done by E. Brömstein (*Ber.*, 1901, **34**, 1268–74).

The oxidation of aniline hitherto has resulted in products in which the amino group has been attacked, yielding for example, azo, azoxy-, nitroso- and nitrobenzene, phenylhydroxylamine, amino-phenol, and finally quinone. Brömstein oxidises aniline salts in neutral solution at a low temperature and at a certain concentration, preferably with lead peroxide. A 20–25% yield of amino-diphenylquinonediimine, $N_2NC_6H_3(NC_6H_5)_2$ is obtained which on more energetic oxidation is converted into azophenine, $C_6H_2(NHC_6H_5)_2(NC_6H_5)_2$. The conversion of the amino-quinone-imine into azophenine can also be shown by dissolving the former in an excess of aniline and warming the solution for a short time with some aniline hydrochloride or zinc chloride. For the preparation of amino-diphenylquinonediimine, aniline hydrochloride or sulphate is dissolved in 20–25 times its weight of water and oxidised with one and a half to twice its weight of lead peroxide (PbO_2) in the form of paste in the cold. The magenta coloured filtrate contains a dyestuff of no particular value, and the residue after drying is extracted with benzene and treated with light petroleum spirit which precipitates a tarry residue. The solution on standing deposits crystals, which recrystallised from alcohol separate in blackish-red nodules. The residue insoluble in alcohol consists of azophenine, m. p. 246°. Amino-diphenylquinonediimine melts at 167°, and is very unstable towards acids. Reduction with ammonium sulphide yields a colourless substance melting at 83°, which gives an acetyl compound corresponding to the formula: $C_6N_3(NHC_2H_3O)(NHC_6H_5)_2$, m. p. at 171°.

Detection and Separation of Aniline

The foregoing colour reactions are amply sufficient for the recognition of aniline, provided that a proper process of separation be previously applied.

Aniline may be liberated from the aqueous solutions of its salts by addition of sodium hydroxide, and may then be extracted by agitating the liquid with ether. On separating the ethereal layer, and agitating it with dilute hydrochloric acid, the aniline passes into the aqueous liquid, which may then be concentrated or evaporated to dryness, and examined by the colour reactions already described. From strychnine, which is the only substance with which aniline is at all likely to be confounded, it may be separated by adding sodium hydroxide to the concentrated solution, and distilling over the aniline by means of a current of steam. The strychnine remains in the flask, whilst the aniline will be found in the distillate if it be acidified with hydrochloric acid and concentrated to a small volume at 100°. The same plan may be employed for detecting aniline in toxicological cases, or the process used for isolating strychnine may be used, but instead of evaporating the ether-chloroform it should be separated and agitated with dilute hydrochloric acid in the manner above described.

F. Müller (*Trans.*, 1888, **52**, 514) claimed to have found unchanged aniline in the urine of a person poisoned with it. The urine was optically inactive, but reduced Fehling's solution. A portion of the concentrated urine, when boiled with strong hydrochloric acid, neutralised with sodium hydroxide, and extracted with ether, gave an ethereal solution which showed the blue indophenol reaction. Numerous investigators have found that aniline taken into the body is excreted in the form of *p*-amino-phenol and its salts. A direct test for the presence of *p*-amino-phenol sulphates in urine consists in boiling the liquid with one-fourth of its volume of strong hydrochloric acid, adding a few c.c. of a 3% solution of phenol, and then some drops of a chromic acid solution. If *p*-amino-phenol be present, the liquid becomes red, and turns blue on adding ammonia.

The *estimation* of aniline may be effected by evaporating its ethereal solution, or preferably by extracting the base therefrom by agitation with dilute hydrochloric acid, evaporating the acid liquid, and weighing the residual hydrochloride. In favourable circum-

stances it may be measured after liberation from a strong solution of the hydrochloride by addition to alkali hydroxide.

Instead of weighing the aniline hydrochloride, the salt may be redissolved in water, and the solution titrated with standard silver nitrate. Or it may be titrated with standard alkali hydroxide and phenolphthalein or litmus, as aniline hydrochloride acts on these indicators exactly like an equivalent quantity of free hydrochloric acid, and the end-point is sharply marked. The process allows of the titration of aniline in presence of neutral ammoniacal salts. On the other hand, with helianthin (Methyl Orange), the basic character of free aniline is distinctly marked, but the end-point is not sufficiently definite to render the indicator available for accurately titrating aniline.

According to Julius (*J. Soc. Dyers, etc.*, **21**, 79) free aniline in aqueous solution can be satisfactorily titrated with standard sulphuric or hydrochloric acid if Congo Red be employed as an indicator and the neutral point be regarded as that at which a bluish-violet colour is obtained, not changed by further small additions of acid; but a much larger excess is required to produce a pure blue. Results are said to be obtainable agreeing within 0.2% with the theoretical.

Probably the most convenient and reliable method for estimating aniline is the titration with bromine the standard $KBrO_3$-KBr solution being used in the presence of acid. This method will be described more fully under aniline oil.

Salts of Aniline

Aniline combines readily with acids, forming a series of salts which crystallise well. The following are the most important.

Aniline Hydrochloride.—$C_6H_5NH_2.HCl$. This salt crystallises with great facility in colourless needles or large plates, which are very soluble in water and alcohol. M. p. 198°, b. p. at 245° unchanged. It yields double salts with stannic, mercuric, antimonious, platinic and auric chlorides; *aniline chloroplatinate*, $(C_6H_5NH_2HCl_2)PtCl_4$ crystallises from hot water in yellow needles. *Aniline salt* is the ordinary commercial name for aniline hydrochloride. It is manufactured by mixing the calculated weights of aniline and hydrochloric acid in stone-tanks, freeing the crystals formed from the mother-liquor by a centrifugal machine, and drying them. According to another process, aniline is dissolved in petroleum spirit of 0.720 sp.

gr., and hydrochloric acid gas passed in until the solution is saturated. The aniline salt is deposited as a white powder, which is separated from the adhering petroleum spirit by hydraulic pressure, and ground to a powder.

Aniline salt is employed largely in calico-printing, its chief use being for the production of *aniline-black* (Vol. VI). It is important that the salt intended for this purpose should be made from pure aniline, and should be dry and neutral. The presence of free acid in the aniline salts is liable to cause the cloth dyed black to rot in the steaming process. It must be free from sand or grit, which would injure the printing rollers, and would produce streaks on the printed cloth. *Grit* remains undissolved when the sample is treated with hot water, and may be filtered off, dried or ignited, and weighed. *Free acid* is best estimated by titration with N/10 sodium hydroxide, with Methyl Orange as an indicator, but the results are not very satisfactory. A useful method of examination consists in titrating the aqueous solution of 2 grm. of the sample with normal sodium hydroxide, with litmus or phenolphthalein as an indicator. The amount neutralised corresponds to the total acid, both free and combined with aniline. Theoretically, 2 grm. of pure aniline hydrochloride would require 15.4 c.c. of normal sodium hydroxide, but owing to the presence of toluidine and moisture, commercial samples of good quality require between 14 and 15 c.c.[1] The process will indicate the presence of ammonium chloride, which will not neutralise alkali, and hence a sample containing it will require a less volume of the standard solution. *Ammonium chloride* is occasionally met with in considerable proportion as an adulterant of aniline salts. For its accurate estimation the sample should be dissolved in water, excess of sodium hydroxide added, the liberated aniline separated, and the aqueous solution distilled in the usual way. On titrating the distillate with standard acid and Methyl Orange, only the ammonia will be indicated. *Fixed impurities* will be detected on igniting the sample; only a mere trace should be present. An idea of the proportion of *toluidine* present in the sample can be obtained by liberating the mixed bases from the solution of the salts by sodium hydroxide, and heating a few c.c. of the aniline with an equal quantity of strong arsenic acid solution to 180° for some time. On boiling the product with water, the intensity of the crimson

[1] This method of examining aniline salts is due to R. Williams (*Chem. News*, 50, 299), but he appears to attribute the reaction to the presence of free acid.

coloration will increase with the proportion of toluidine in the sample.

Aniline Sulphate, $(C_6H_5NH_2)_2H_2SO_4$. –This salt forms a crystalline powder, which is readily soluble in water and slightly so in alcohol. It is insoluble in ether, a fact which distinguishes it from the sulphate of methylamine.

Aniline Phosphate, $(C_6H_5NH_2)_2H_3PO_4$, crystallises in plates. It is easily soluble in water, ether and hot alcohol, slightly soluble in cold alcohol.

Aniline Oxalate, $(C_6H_5NH_2)_2H_2C_2O_4$, is easily soluble in water, soluble with difficulty in absolute alcohol, and insoluble in ether.

Aniline Acetate, $C_6H_5NH_2.HC_2H_3O_2$, does not appear to have been obtained in a definite crystalline form. When heated it loses the elements of water and forms acetanilide.

Aniline-sulphonic Acids. Amino-benzene-sulphonic Acids

When aniline is treated with an equivalent amount of dilute or concentrated sulphuric acid it is converted into aniline sulphate. If an excess of acid be used, a high temperature employed, or sulphuric anhydride be present, aniline-sulphonic acid is produced:

$$C_6H_5.NH_2 + SO_2(OH)_2 = C_6H_4\begin{cases} SO_2.OH \\ NH_2 \end{cases} + H.OH$$

Three modifications of this substance exist, which differ according to the relative positions of the NH_2 and SO_3H groups in the benzene-nucleus. The ortho-sulphonic acid (1:2) has no practical interest, but the meta- and para-acids are manufactured on a large scale for the production of azo-dyes.

m-Amino-benzene-sulphonic acid, $C_6H_4(NH_2)^{(1)}.SO_3H^{(3)}$, is employed for the manufacture of *Metanil-yellow* (Vol. 6). It is prepared by warming nitrobenzene with fuming sulphuric acid, or by treating a solution of benzene in strong sulphuric acid with fuming nitric acid, when a mixture of nitro-benzene-sulphonic acids, $C_6H_4(NO_2)SO_3H$ is obtained, in which the meta-acid predominates, and may be roughly separated from its isomers by conversion into the barium or calcium salt. The meta-nitro-sulphonic acid yields, on reduction, the corresponding amino-sulphonic acid.

p-Amino-benzene-sulphonic acid, $C_6H_4(NH_2)^{(1)}.SO_3H^{(4)}$, likewise called *Sulphanilic Acid*, is prepared by heating 1 part of aniline and 3 of concentrated sulphuric acid to 195°. With fuming acid, the reaction occurs more rapidly and at a lower temperature. On pouring the cooled product into water, the acid separates as a crystalline mass, which can be recrystallised from hot water.

Sulphanilic acid crystallises in rhombic tables containing 1 H_2O, which effloresce in the air, and are only slightly soluble in cold, but readily in hot, water. Treatment with potassium dichromate and sulphuric acid oxidises it to quinone, $C_6H_4O_2$. The solution of the sodium salt, on treatment with sodium nitrite, yields sodium diazobenzene-sulphonate. Sulphanilic acid in the commercial product may be estimated by titration with standard sodium nitrite solution with starch potassium iodide paper as an indicator. The product should be examined for free sulphuric acid and for aniline sulphate.

Dissolve 10 g. of the powdered sample in 200 c.c. of water containing 5 grm. of sodium hydroxide. If aniline sulphate is present, upon distilling this solution with steam free aniline will be found in the distillate. Aniline sulphanilate loses all its basic properties at 100°.

Nitranilines.—When aniline is treated with dilute nitric acid it yields nitraniline. With the concentrated acid it reacts far more violently than benzene, and is converted into quinone and other products. To obtain a nitro-derivative by such means, the aniline must be protected by employing its acetyl-derivative, or by nitrating in presence of an excess of strong sulphuric acid. In the latter case a mixture of the 3 isomeric nitranilines is obtained, but chiefly the *meta*-compound; in the former case *para*-nitracetanilide, $C_6H_4(NO_2).NH(C_2H_3O)$, is formed, together with some of the *ortho*-compound, both of which readily yield the corresponding nitraniline, $C_6H_4(NO_2).NH_2$, on boiling with concentrated hydrochloric acid or alkali hydroxide.

Another method of preparing the nitranilines, especially the *meta*-modifications, is the reduction of the corresponding dinitrobenzenes in alkaline alcoholic solution. In these circumstances only one of the NO_2 groups is reduced to NH_2, whereas in acid solution diamino-benzene, $C_6H_4:(NH_2)_2$, is obtained.

	Nitranilines, $C_6H_4(NO_2).NH_2$		
Appearance and Crystalline form	*Ortho.* $NO_2 : NH_2 = 1 : 2$	*Meta.* $NO_2 : NH_2 = 1 : 3$	*Para.* $NO_2 : NH_2 = 1 : 4$
	Orange-yellow needles.	Long yellow needles.	Long yellow needles.
Taste	.. .. ..	Sweet, burning.	Nearly tasteless.
M. p	71.5°	110°	147°
Volatility	Distils in a current of steam.	Sublimes at 100°. Distils in a current of steam.	Not volatile with steam.
Salts	Very unstable.	Fairly stable.	Unstable.
Behaviour when boiled with strong sodium hydroxide.	.. .. ..	Unchanged.	Forms *p*-nitrophenol—$C_6H_4(NO_2).OH$.

The nitranilines are yellow crystalline substances, readily soluble in alcohol, but only slightly so in water. They are weak bases, forming yellow salts, and yield the corresponding diamino-benzenes on reduction. The preceding table shows their chief differences.

Commercial *p*-nitroaniline is examined by determining its m. p., and testing for the presence of the meta isomer, a test for which is made as follows: Heat 0.25 grm. of the sample with zinc and hydrochloric acid until the solution is colourless. Filter, dilute to 250 c.c. To 10 c.c. of the solution diluted to 50 c.c. add 2 or 3 drops of dilute sodium nitrite solution. A pale yellow colour is produced if the sample is free from the meta compound, but brownish if the meta compound is present, due to the formation of Bismark Brown. The *p*-nitroaniline content may be accurately estimated by titration with N/2 sodium nitrite solution, with starch iodide paper as indicator, or by coupling with β-naphthol.

There are 6 *dinitranilines*, $C_6H_3(NO_2)_2.NH_2$, melting as follows: 2–6, 138°; 2–4, 176°; 2–3, 127°; 2–5, 137°; 3–4, 154°; 3–5, 159°. Also a *trinitraniline*, $C_6N_2(NO_2)_3.NH_2$, or picramide, m. p. 188°, and is converted into picric acid, $C_6H_2(NO_2)_3.OH$, and ammonia when boiled with alkali hydroxide.

Homologues of Aniline

As already stated, the true homologues of aniline are substances in which 1 or more atoms of the hydrogen of the benzene nucleus are replaced by a corresponding number of methyl or other alkyl radicals. The compounds in question may be prepared, and are produced commercially, by processes exactly similar to those which result in the formation of aniline. That is, the hydrocarbons tolu-

ene, xylene, etc., are treated with nitric acid, and the resulting nitro-derivatives are reduced to the bases by nascent hydrogen (usually produced from iron and hydrochloric acid).

In their general chemical relationships the homologues present the closest resemblance to aniline, and yield substitution products of a strictly parallel character. They are also diazotised similarly.

The only homologues of aniline which require separate descriptions are the toluidines, $C_6H_4CH_3NH_2$, and the xylidines, $C_6H_3(CH_3)_2NH_2$. Their consideration will be followed by a section describing "aniline oils," under which term are included commercially pure aniline and toluidine and various mixtures of these bases.

Toluidines.—Amino-toluenes. Amino-methylbenzenes.

$$C_6H_4.CH_3.NH_2$$

The toluidines exist in small quantity, together with aniline, in coal-tar. They are produced commercially from toluene by processes exactly analogous to those by which aniline is prepared from benzene, and together with aniline constitute nearly the whole of the "aniline oils" of commerce.

Three isomeric modifications of toluidine are known. The chief physical differences between them are shown in the following table, in which they are also contrasted with aniline and their isomeride benzylamine, $C_6H_5.CH_2NH_2$.[1]

	Aniline	*o-Toluidine* $CH_3{:}NH_2 = 1{:}2$	*m-Toluidine* $CH_3{:}NH_3 = 1{:}3$	*p-Toluidine* $CH_3{:}NH_2 = 1{:}4$	*Benzyl-amine*
Sp. gr. at 15°......	1.0268	1.0037	0.998 (at 25°)	Solid.	0.990
M. p.	Solidifies at −8°	Does not solidify at −20°	Does not solidify at −13°	+45°	Liquid.
B. p.	184°	199.5°	197°	198°	185°
Characters of the acetyl derivative: M. p. . .	114°	107°	65–66°	147°	57–61°
B. p.	295°	296°	302–304°	307°	300°
100 pts. of water dissolve . . .	3.4 at 14°	8.6 pts. at 19°	4.4 pts. at 13°	0.89 at 22°	Soluble.
Solubility of the acid oxalate: In 100 pts. of water at 15°. . . .	. . . .	23.8	26.5	8.7	. . . .
In 100 pts. of ether at 15°.	. . . .	0.50	Very slight.	6	. . . . . .

[1] BENZYLAMINE is a colourless liquid of faint aromatic odour, and is not affected by light. It is miscible in all proportions with water, alcohol and ether, but is separated from its aqueous solutions by alkali hydroxides (compare "Pyridine"). It has a strongly alkaline reaction, fumes with hydrochloric acid, and absorbs carbon dioxide from the air, with conversion into silky needles of the *carbonate*.

Ortho-toluidine is formed by the reduction of *o*-nitrotoluene. It is a colourless liquid, turning brown on exposure to air or light, and otherwise closely resembling aniline. It differs from its isomerides by giving a green coloration when treated with ferric chloride and a little para-diamino-benzene. A solution of 1 in 10,000 gives a fairly deep coloration, and one of 1 in 100,000 assumes a distinct greenish tint. All commercial aniline gives this coloration, and even that prepared by the distillation of indigo with alkali hydroxide.

Meta-toluidine is produced by the reduction of *m*-nitrotoluene. It is only present in small proportion in commercial toluidine. For its detection and approximate estimation the mixed bases are converted into hydrochlorides, and the greater part of the isomeric salts removed by fractional crystallisation. The mother-liquor is evaporated to dryness, and the residue heated with methyl alcohol to 200°, under pressure, for a considerable time. This produces a mixture of the three isomeric dimethyl-toluidines, but only the meta-modification yields a nitroso-derivatives, $C_6N_3(NO)(CH_3).N(CH_3)_2$, on adding sodium nitrite to an ice-cold solution of its hydrochloride. The hydrochloride of nitroso-dimethyl-*m*-toluidine thus prepared, crystallises from a hot acidified solution in greenish-yellow needles only slightly soluble in cold water. On treatment with sodium carbonate the free base is obtained, m. p. 92°, crystallising from water or ether in small green plates or long needles, and precipitated in moss-green needles on adding petroleum spirit to its chloroform solution. All its solutions have a deep green colour. Nitrosodimethyl-*m*-toluidine forms steel-blue compounds with aniline and *o*-toluidine.

A detailed study of the oxidation products of *p*-toluidine has been made by E. Brömstein (*Ber.*, 1901, **34**, 1274–84), and a resumé of the work is here given.

According to Rosenstiehl, the 3 modifications of toluidine may be distinguished by the following reactions:

	o-Toluidine	*m-Toluidine*	*p-Toluidine*
1. To a solution of the base in sulphuric acid, of 1.75 sp. gr., add a solution of chromic acid in sulphuric acid of the same strength.	Blue coloration changing on dilution to a permanent red-violet.	Yellow-brown coloration, becoming greenish-yellow on slight dilution, and colourless on further addition of water.	Yellow coloration.
2. To a solution of the base in sulphuric acid of 1.75 sp. gr., add nitric acid.	Orange colouration, or in very concentrated solutions, brown, becoming yellow on dilution.	At first red, rapidly changing to intense blood-red, and then dirty red; on dilution, orange.	Blue streaks which soon tinge the whole liquid; (in presence of aniline or *o*-toluidine, blood red). The colour quickly becomes violet, then red, and, after some hours, brown.
3. Dissolve the base in ether, and add an equal volume of water. Then add a few drops of clear solution of bleaching powder.	The aqueous layer becomes first yellow and then brown. The ethereal layer, after separation, gives a permanent reddish-violet coloration with dilute sulphuric acid.	The aqueous layer becomes a thick brownish-yellow. The ethereal layer becomes reddish, and after separaton and addition of dilute sulphuric acid is coloured violet at the under-surface.	No reaction. In presence of aniline the ether becomes blue on agitation

p-Toluidine is produced by the reduction of the nitrotoluene derived from the toluene produced by the dry distillation of Tolu balsam; also by heating *p*-cresol to 300° with ammonia and zinc chloride; and by molecular transposition from methylaniline hydrochloride. It crystallises from hot dilute alcohol in colourless plates melting at 45°, and has a peculiar odour recalling that of aniline. Commercially it is now produced from pure crystalline para-nitrotoluene separated from commercial nitrotoluene by sweating, and vacuum distillation, by reduction with iron powder and hydrochloric acid.

In 1880 W. H. Perkin, by the action of potassium dichromate on *p*-toluidine sulphate, obtained 2 oxidation products, having the formulas $C_{21}H_{21}N_3$ and $C_{28}H_7N_3$, respectively. The latter compound was investigated by later workers, its constitution being determined by A. G. Green (*J. Soc. Chem. Ind.*, 1894, **13**, 143), who showed it to be an amino-ditolyl-*p*-toluquinonediimine. Perkin applied the oxidation with lead peroxide in similar manner to that employed in the case of aniline and obtained according to the conditions one or other of Perkin's compounds.

By oxidising *p*-toluidine hydrochloride or sulphate with lead peroxide (2½–3 times the weight of the hydrochloride) in a dilution of 10–60 times, in the former case Perkin's[1] and in the latter Barsilow-

[1] Perkin's base (*p*-tolylamino-ditolyl-*p*-toluquinonediimine).

sky's[1] base is obtained. A 6% yield of the latter is produced by dissolving 100 grm. of *p*-toluidine in 1 litre of water, together with just the necessary quantity of hydrochloric acid, and oxidising with 587 grm. of manganese peroxide paste (15%). There were recovered 4 grm. of *p*-toluidine and 9.6 grm. of peroxide, so that 96 grm. of toluidine or 128 grm. of the hydrochloride required 78 grm. of manganese peroxide or in the proportion of 10:6. No Perkin's base was obtained, but 6 grm. of Barsilowsky's base and a considerable amount of azotoluene. An 18% yield of Perkin's base was obtained by dissolving 218 grm. of *p*-toluidine and 98 grm. of sulphuric acid, in 6.5 litres of water and adding 294 grm. of potassium dichromate in 35 litres of water. After 24 hours the dark brown precipitate was filtered off, dried, and extracted with benzene, which, after evaporation, yielded the mixture of bases. On boiling with 20 times the weight of absolute alcohol, 39 grm. of Perkin's base remained behind, while only 0.2 grm. of Barsilowsky's base was formed. In another preparation, 267.5 grm. of *p*-toluidine gave 54 grm. or 20% of Perkin's base, and 3 grm. of Barsilowsky's base. The latter melts at 235° and dissolves in concentrated sulphuric acid, with a pure blue colour. As already stated, on boiling this base with *p*-toluidine and its hydrochloride in alcoholic solution it is converted, not into Perkin's base, but into the hydrochloride of the product $C_{35}H_{35}N_5$, which crystallises in brassy yellow plates, melting at 282°. The base separates from dilute alcohol in orange-red plates melting at 251°. Perkin's base, or tolylaminoditolytoluquinonediimine, melts at 183°, and dissolves with a violet colour in concentrated sulphuric acid, which turns green, blue, and then violet-red on dilution with water. The compound is basic and dissolves with a violet-red colour in hydrochloric acid, but the salts are easily decomposed. On treatment with 20 times its weight of 5% alcoholic sulphuric acid and standing 24 hours, the purple red solution passes through violet to pure blue. On saturation with ammonia and recrystallisation from ethyl or methyl alcohol, brownish-red shining needles melting at 181°, are obtained, the yield being 75% of the theoretical. The solution in concentrated sulphuric acid is green, becoming more and more reddish on standing and turning deep orange-yellow on dilution with water.

[1] Barsilowsky's base (amino-ditolyl-*p*-toluquinonediimine).

Commercial toluidine, so-called "mixed toluidine," consists of the normal mixture obtained by nitrating and reducing pure toluene and contains about 34% of the para, 62% ortho and 4% of the meta compound. According to Friswell, the sp. gr. of the *o-toluidine* of commerce should be about 1.0037, and its b. p. from 197 to 198°. It ought not to solidify on cooling to $-4°$, though the majority of samples contain sufficient *p*-toluidine to cause them to commence crystallising at this temperature. The *p-toluidine* of commerce occurs in white dry crystals, m. p. 43–45°, and distils between 196 and 198°. *Liquid* commercial toluidine should boil at 197–198°, and have a sp. gr. of about 1.000.

A portion of the *p*-modification separates from the commercial mixture of the isomers when the liquid is cooled by a freezing mixture. A further separation is effected in practice by fractionally saturating the mixture of the bases with sulphuric acid, and then distilling in a current of steam. *o*-Toluidine, being a weaker base than the para-compound, will alone pass into the distillate if the quantity of sulphuric acid employed be somewhat in excess of that requisite to neutralise the *p*-toluidine.

L. Lewy (*Trans.*, 1887, **50**, 872) has proposed to separate *o*- and *p*-toluidine by converting the bases into phosphates. It appears that when *p*-toluidine and orthophosphoric acid are brought together, *di*-toluidine orthophosphate, $(C_6N_4CH_3NH_2)_2H_3PO_4$ is produced as a salt crystallising in scales and very sparingly soluble in cold water, but more readily, with partial dissociation, in boiling water. Aniline acts similarly, forming a sparingly soluble *di*-aniline orthophosphate, $(C_6H_5NH_2)_2H_3PO_4$. On the other hand, *o*-toluidine forms a *mono*-toluidine orthophosphate, $(C_6H_4CH_3NH_2)H_3PO_4$, and never a di- or tri-salt. Hence in the phosphates obtained from a mixture of the 2 toluidines the proportions of the bases might be deduced from the percentage of phosphoric acid. The mono-orthotoluidine phosphate is more readily soluble in water than diparatoluidine or dianiline phosphate. Further, when its solution is shaken with free aniline or *p*-toluidine, the *o*-toluidine is set free. Hence pure *o*-toluidine can be obtained from commercial toluidine[1] by adding rather more of a 21% aqueous solution of phosphoric acid than will suffice to form diphosphates with the aniline and *p*-toluidine present. On warming the liquid, the free *o*-toluidine forms a layer at the sur-

[1] The xylidines and cumidines behave like o-toluidine, and form only monophosphates.

face, which may be separated and distilled. The process may be modified by adding a further quantity of phosphate to convert the *o*-toluidine into monophosphate, and then cooling the liquid and allowing it to stand to secure the complete deposition of the *p*-toluidine phosphate.

Wölfing (*Ber.*, 1886, **19**, 2132) states that *o*-toluidine prepared by Lewy himself by the above process, both on the small and large scale, still contained as much as 4% of *p*-toluidine. For the preparation of pure *p*-toluidine he recommends (*Dingl. polyt. J.*, **263**, 260) that the hydrochlorides of the bases should be treated with an amount of sodium nitrite only sufficient to convert the *o*-toluidine present into amino-azotoluene. Only when this change is complete does the *p*-toluidine react with the nitrite to form a diazo-amino-compound.

A method of estimating the proportions of the ortho- and para-modifications of toluidine in the commercial product has been based by Rosenstiehl on the different solubilities of the acid oxalates of the two bases. The acid oxalate of *p*-toluidine requires 6660 pts. of ether for solution, whilst the corresponding salt of *o*-toluidine dissolves in 200 pts. of ether. The method, somewhat modified, is as follows: 0.2 grm. of the sample is dissolved in 80 c.c. of anhydrous ether free from alcohol; 1.059 grm. of anhydrous oxalic acid, or 1.177 grm. of the crystallised acid, is dissolved in 250 c.c. of anhydrous, alcohol-free ether. Each c.c. of this solution will precipitate 0.005 grm. of toluidine. An excess is added to the ethereal solution of the sample, the liquid allowed to stand in a stoppered bottle for 12 hours, then filtered through paper and the precipitate washed with ether. The precipitate is then washed into the bottle with water, and the solution titrated with N/10 alkali hydroxide and phenolphthalein. 1 c.c. of N/10 alkali represents 0.00535 grm. of *p*-toluidine. Miniati, Booth and Cohen (*J. Soc. Chem. Ind.*, 1889, **6**, 419) find that if too long a time be allowed for the precipitation, the product is liable to contain the *o*-toluidine oxalate, and hence the result will be high. They recommend that a repetition of the proceedure should be made, in which the amount of oxalic acid solution used is only that requisite to combine with the *p*-toluidine found by the first test, so reducing the error to a minimum.

G. Lunge (*Chemische Ind.*, **8**, 74) estimates the proportion of *p*- and *o*-toluidine in a mixture of the two by a careful observation of the sp.

gr. The determination is made by the pycnometer, and referred to water at 15°. If the sample does not contain more than 50% of *p*-toluidine it is liquid at 15°, and consequently the observation is made at that temperature. With 50 to 60% of *p*-toluidine the method is still available if the pycnometer is filled at 20°; but with still larger proportions the results are unreliable, as the correction for temperature loses in accuracy, and the differences in sp. gr. become very small for considerable alterations in the composition of the mixture. It is very desirable to adhere rigidly to the prescribed temperature, as an error of 1° causes an error of 7% in the estimation. The correction is ±0.0008 for 1°, when the sp. gr. is above 1.0008 and ±0.0007 when below that point. All water must be removed by treating the sample with powdered potassium hydroxide and redistilling. The distillation also serves to show the presence of aniline or xylidine, in presence of notable quantities of which the method is inapplicable.

Lunge gives the following table of densities of mixtures of *p*- and *o*-toluidine, water at 15° being taken as unity:

Sp. gr. at 15°	*o*-Toluidine, %	Sp. gr. at 15°	*o*-Toluidine, %	Sp. gr. at 20°	*o*-Toluidine, %	Sp. gr. at 20°	*o*-Toluidine, %
1.0037	100	1.0016	82½	0.9995	65½	0.9939	50
36	99	15	82	94	65	38	49½
35	98	14	81	93	64	37	48½
34	97	13	80	92	63	36	48
33	96	12	79½	91	62	35	47½
32	95	11	78½	90	61½	34	46½
31	94	10	77½	89	61	33	46
30	93½	09	77	88	60	32	45
29	92½	08	76	87	59	31	44½
28	91½	07	75	86	58½	30	44
27	91	06	74	85	58	29	43
26	90	05	73	84	57½	28	42
25	89½	04	72½	83	56½	27	41
24	88½	03	72	82	56	0.9926	40
23	88	02	71	81	55	....	....
22	87	01	70	80	54½	....	....
21	86½	1.0000	69	79	54	....	....
20	86	0.9999	68½	78	53	....	....
19	85	98	68	77	52½	....	....
18	84½	97	67	76	51½	....	....
1.0017	83½	0.9996	66½	0.9975	51	....	....

A method of separating *o*-toluidine from *p*-toluidine has been based by P. Schoop (*Chem. Zeit.*, 1885, **9**, 1785) on the observation of Weith and Merz, that the acetyl derivative of *o*-toluidine is far less soluble in water than that of the isomer and of aniline. Schoop's method has been found unsatisfactory by several chemists, and need not be further described.

A method of estimating *p*-toluidine in admixture with *o*-toluidine has been based by G. A. Schoen (*Chem. Zeit.*, **12**, 494; *J. Soc. Chem. Ind.*, **7**, 594) on the intensity of the red colour produced with potassium dichromate. If the sp. gr. indicates the presence of more than 8% of *p*-toluidine it is reduced below that proportion by adding *o*-toluidine. 1 c.c. of the oil is then dissolved in 2 c.c. of hydrochloric acid and 30 of water, and 1 c.c. of a cold saturated solution of dichromate of potassium added. The mixture is allowed to stand for an hour, with occasional stirring, and is then filtered. *o*-Toluidine gives a black lake and a colourless liquid, but in presence of *p*-toluidine the precipitate is light brown, and the filtrate has a red colour, intense in proportion to the *p*-toluidine present. Pure aniline behaves like *o*-toluidine, but in the presence of the latter a red filtrate is produced. Hence aniline must be absent, or its amount must be deduced from the b. p. and sp. gr. of the sample, and a corresponding amount added to the standard mixture with which the sample is compared.

A method of estimating small quantities of impurities in *o*-toluidine and *o*-nitrotoluene has been proposed by A. F. Holleman. (*Rec. trav. chim. Pays-Bas*, 1908, **27**, 458–642.) The impurity usually encountered in *o*-toluidine is the *p*-compound, and the amount of this is estimated by converting the sample into the acetyl derivative and observing the solidifying point. The solidifying points of known mixtures of the acetyl compounds of *o*- and *p*-toluidine are given in the following table:

p-Compound, %	Solidifying point
0	109.15°
1.12	108.45°
2.42	107.75°
9.58	103.2°
13.6	100.8°

42.8 grm. of the toluidine to be tested are added slowly to a solution of 25.2 grm. of oxalic acid in 1 litre of hot water. After cooling

to 0°, the crystals are collected on a filter and washed once with a little water. The toluidine is then regenerated from both crystals and filtrate by adding sodium hydroxide and distilling in steam. After separating the oil, the aqueous liquor must be extracted twice with ether to avoid loss. Both portions of toluidine are now converted into the acetyl compound by using for 1 grm. of toluidine, 2 c.c. of glacial acetic acid and 1 c.c. of acetic anhydride, evaporating on a water-bath and distilling in a vacuum. The solidifying point of the 2 portions is determined and the amount of *p*-toluidine deducted from above table. When the amount of *p*-compound exceeds 1–2% the toluidine can be directly converted into the acetyl derivative without first preparing the oxalate. The presence of *p*-nitrotoluene in *o*-nitrotoluene is detected by first reducing with iron and hydrochloric acid and treating the resulting toluidine in the above manner.

The method of estimating the toluidine content by means of titration with $KBrO_3.KBr$ standard solution will be found valuable in the examination of commercial toluidines. Both *o*- and *p*-toluidine form dibrom-compounds. Whilst the *m*-toluidine forms the tribromn compound. Dissolve 5 grm. of the sample in 100 c.c. concentrated HCl and 100 c.c. of water and make up to 500 c.c. Titrate 25 c.c. aliquot parts with N/10 $KBrO_3.KBr$ solution, using a 2-minute end point with starch iodide paper. A distinct yellow colour will be imparted to the solution by the slightest excess of $KBrO_3.KBr$ solution and this may be used in checking the end point. The standard $KBrO_3.KBr$ solution should be standardised against pure *o*- or *p*-toluidine.

Commercial *o*-toluidine is examined for the distillation range, colour, solubility in hydrochloric acid, moisture and toluidine content. *p*-Toluidine is examined for freezing point, solubility in hydrochloric acid, colour, moisture and total toluidine.

Xylidines.—Amino-dimethylbenzenes. $C_6H_3(CH)_2.NH_2$.

Six isomeric substances of the above formula are theoretically possible, and all of them are known. Thus:[1]

[1] The table is chiefly drawn up from the descriptions of the isomeric xylidines given by Roscoe and Schorlemmer. The characters diffei considerably from those attributed to the isomers by Wroblewsky (*Annalen*, **207**, 91). Nölting and Pick (*Ber.*, 1888, **21**, 3150), however, consider that Wroblewsky's *v*-*o*-xylidine was simply impure metaxylidine, and give the following table of characters of xylidine salts:

	Orthoxylidine	Orthoxylidine	Metaxylidine	Wroblewsky's so-called Orthoxylidine
Hydrochloride	$+1H_2O$	$+1H_2O$	$+\frac{1}{2}H_2O$; needles	$+\frac{1}{2}H_2O$
Solubility in 100 of water at 18°	11.2	Very soluble.	9.2	Very soluble.
Nitrate	Anhydrous.	Anhydrous.	Anhydrous.	Anhydrous.
Solubility in 100 of water at 18°	6.6	0.4	2.2	2.7
Normal sulphate	Anhydrous.	Anhydrous.	Anhydrous.	Anhydrous.
Solubility in 100 of water at 18°	1.4	5.6	Very soluble.	....
Acid sulphate	Is not formed under ordinary conditions.		$+2\frac{1}{2}H_2O$	$+2\frac{1}{2}H_2O$
Solubility in 100 of water at 18°	....	....	6.2	Very soluble

Base	Positions of Groups $CH_3:CH_3:NH_2$	B. p.	Acetyl derivative		Characters of Hydrochloride
			M. p.	Appearance, etc.	
v-Orthoxylidine	1:2:3	223°	134°	White needles.	Moderately soluble white needles containing 1 H_2O.
a-Orthoxylidine	1:2:4	226° (melts at 49°)	99°	Long vitreous prisms.	Long, very thin prisms, containing 1 H_2O.
v-Metaxylidine	1:3:2	216°	176.8°	White needles; not saponified by boiling alkali or acid.	Thin anhydrous plates; readily soluble
a-Metaxylidine	1:3:4	212°	129°	White needles.	Anhydrous rhombic tablets; slightly soluble in cold water.
s-Metaxylidine	1:3:5	220°	140.5°	Large flat needles.	Large anhydrous needles.
Paraxylidine	1:4:2	213.5°	139°	Long lustrous needles.	Flat needles or large tablets.

The modifications of xylidine produced by nitrating the xylenes of coal-tar naphtha and reducing the nitro-derivatives are chiefly *α-o*-xylidine, *α-m*-xylidine, and *p*-xylidine, but 2 of the other isomers are also said to be produced. Only the *a*-meta-modification is of value for the manufacture of azo-colouring matters, and of the cumidines, $C_6H_2(CH_3)_3.NH_2$, which are prepared by heating xylidine hydrochlorides with wood alcohol, only pseudo-cumidine is of value. On this account, the useless isomers are removed as far as possible from

the metaxylene before nitrating, and in fact the presence of even a few units per cent. of *o*-xylene will occasion considerable practical inconvenience by the formation of tarry matters during its conversion into xylidine. On the other hand, commercial xylidine often contains as much as 25% of *p*-xylidine. *v*-*m*-xylidine (1:3:2) is prepared by converting commercial xylidine into the sulphate, which is allowed to crystallise, and the base liberated from the mother-liquor by alkali. The fraction distilling between 212° and 216° is heated with acetic anhydride. The *v*-*m*-acetxylidide formed is not acted on by boiling for several hours with 4 times its weight of dilute sulphuric acid containing 25% of H_2SO_4, but its isomers are decomposed. On cooling, the unchanged acetyl-compound separates, and after recrystallisation from hot water melts at 176.8°.

On heating it for some time to 200° with three parts of sulphuric acid containing 70% of H_2SO_4, the sulphate of *v*-*m*-xylidine is formed. This salt differs from the sulphate of the isomeric xylidines in its very ready solubility in water.

ANALYSIS OF COMMERCIAL XYLIDINE[1] (FLOTATION GRADE). $C_6H_3(CH_3)_2.NH_2$ M.W.121.13.

1. *Moisture.*—Weigh 200 grm. of the sample into a 500 c.c. distilling flask (with outlet tube 1 in. below top of neck). Add 100 c.c. water-saturated toluol and distil out the water and toluol into a 120 c.c. separatory funnel, with stem graduated in 0.1 c.c. divisions. Measure the water as the lower layer.

$$\frac{\text{c.c. water} \times 100}{200} = \%\ \text{Moisture.}$$

2. *Solubility.*—Weigh a 200 grm. sample into a one litre round bottom distilling flask (with outlet tube 1″ below top of neck), add 200 c.c. water, then C. P. HCl acid until the reaction is distinctly acid to Congo Red paper. Distil over 100 c.c. into a 120 c.c. separatory funnel with upper and lower stem graduated in 0.1 c.c. divisions. If the oil remains suspended throughout the water, add salt to the water. Make certain that the distillate is distinctly acid and measure the oil layer.

$$\frac{\text{c.c. oil} \times 100}{200} = \%\ \text{Insoluble.}$$

[1] Method of the Newport Company—Carrollville Laboratory.

3. *Ash.*—Pipette a 5 c.c. sample into a tared porcelain crucible and weigh. Ignite until free of carbonaceous matter and weigh again.

$$\frac{\text{Wt. of residue} \times 100}{\text{Wt. of sample}} = \% \text{ Ash.}$$

Distillation.—Measure in a graduated cylinder 100 c.c. sample pour into a dry 200 c.c. M. C. A. Pyrex distilling flask and do not rinse out the cylinder. Support the flask on a 6″ × 6″ asbestos board 1/8″ thick with a hole in the center 1¼″ in diameter. Support the board on a circular shield enclosing the flame. Insert, through a cork stopper, a Tykos 14″ thermometer with the top of the auxiliary bulb opposite the middle of the vapour tube. The thermometer shall be graduated in 1/5°, have a range 200°–250° and be standardised totally immersed. Heat the flask with the short flame of a Bunsen burner. Distil at the rate of 1 drop per second through a straight air-cooled condenser tube 24″ long and ½″ internal diameter so placed that the vapour tube will extend 1″ into the narrow portion. Receive the distillate in the same cylinder used for measuring the sample.

Note the temperature at 2 c.c. and 97 c.c. and apply the following corrections:

(*a*) Correction on thermometer, determined by standardisation.

(*b*) Stem correction determined from the equation

$$C = E(T - t)0.00016$$

in which

E = Degrees from centre of stopper to point of reading.

T = Temperature reading.

t = Room temperature surrounding thermometer at point of reading.

C = Stem correction, additive.

(*c*) *Barometer Correction.*—For each m.m. difference between 760 and the observed corrected barometric pressure, add or subtract 0.06°; depending upon whether the observed pressure is below or above 760 m.m.

o-**Xylidine** (1:2:4) is the only modification of xylidine which is solid at ordinary temperatures. By gradually evaporating its solution in petroleum spirit, it is observed in thick monoclinic prisms, but when rapidly deposited, or caused to solidify quickly, it forms trans-

parent vitreous tablets. It melts at 49°, and is sparingly soluble in cold water, but readily in hot water, and also in alcohol and ether. Its aqueous solutions are not coloured by bleaching powder solution. The hydrochloride is readily soluble in water, but only slightly in strong hydrochloric acid; its aqueous solution imparts an intense yellow colour to fir-wood.

m-**Xylidine** (1:3:4), or ordinary xylidine, is best obtained by converting commercial xylidine into the hydrochloride and crystallising the product from water. Both the hydrobromide and hydrochloride are only slightly soluble in cold water. The last traces of impurity can be removed from *m*-xylidine by converting it into the acetyl-derivative, and recrystallising this substance from benzene till it has a m. p. of 129°. It is then decomposed by sulphuric acid.

p-**Xylidine** (1:4:2) has a sp. gr. of 0.980. It is prepared by treating commercial xylidine with fuming sulphuric acid containing sufficient sulphuric anhydride to convert the bases into sulphonic acids. The mixture is heated to 100° for some time, allowed to cool, and the solid mass pressed under water to separate *m*-xylidine-sulphonic acid in the crystalline state; or the hot liquid is poured upon ice, when the *m*-sulphonic acid, being with difficulty soluble in dilute sulphuric acid, crystallises out. The mother-liquor is neutralised with chalk, filtered, precipitated with sodium carbonate, and again filtered. On concentrating the filtrate, the sodium salt of *p*-xylidine-sulphonic acid separates in nacreous plates, which are washed with a little cold water to free them from traces of the readily soluble meta-sulphonate. The salt yields *p*-xylidine on dry distillation with ammonium chloride, whilst the sodium salt of *m*-xylidine-sulphonic acid chars under the same treatment. *p*-Xylidine may also be obtained by nitrating and reducing *p*-xylene, which may readily be prepared from commercial xylene.

Commercial xylidine used in dye manufacture and for floatation is examined for distillation range, solubility in hydrochloric acid, moisture, and xylidine content. The estimation of xylidine is made by weighing about 3 grm. of the sample in a weighing bottle and transferring it to a 2000 c.c. beaker containing a mixture of 25 to 35 c.c. of concentrated HCl in 200 c.c. of water. The bottle is rinsed out with dilute HCl. The solution is stirred to dissolve the xylidine and the solution cooled to about 0 and is diluted with ice and water to about 1000 c.c. Add slight, but known excess of calculated theoreti-

cal N/2 sodium nitrite solution, allow it to stand for 10 minutes, then add known amount of sulphanilic acid in slight excess of amount of excess $NaNO_2$ used. Allow it to stand for about 2 to 5 minutes and titrate excess of sulphanilic acid with N/2 $NaNO_2$ solution to 5 minute end-point, using starch iodide paper.

Cumidines.—Amino-trimethylbenzenes. $C_6H_2(CH_3)_3.NH_2$.

Various isomerides of this formula are known. The solid variety of commercial cumidine is made by heating xylidine hydrochloride and methyl alcohol together under pressure, to about 300°. The bases are liberated and converted into nitrates, and the difficultly soluble nitrate of pseudocumidine separated from the mother-liquor. The base is again liberated and distilled. The fraction passing over between 230 and 240° crystallises on cooling, and consists of amino-pseudocumene: (CH_3:CH_3:CH_3:NH_2 = 1:2:4:5). It crystallises from hot water in long needles, and from alcohol in large prisms, melts at 68°, and boils at 234–236°. When converted into diazocumene it can be used for the preparation of azo-colours by reaction with naphtholmono- and di-sulphonic acids.

Isoduridine. Amino-tetramethylbenzene. $C_6H(CH_3)_4.NH_2$.

When the hydrochloride of pseudocumidine is heated with methyl alcohol to 300°, the hydrochloride of isoduridine is formed. The free base, which also occurs among the by-products of the manufacture of pseudocumidine, is an oily liquid which boils at 250–253°, and solidifies on cooling to crystals, m. p. at 14°.

Amino-pentamethylbenzene. $C_6(CH_3)_5.NH_2$.

This base is obtained by heating dimethyl-α-pseudocumidine with methyl iodide. It forms large white needles, m. p. 151° and b. p. 277°.

Aniline Oils

The term "aniline oils" was formerly applied commercially to all the different varieties of aniline manufactured on a large scale, equally whether the product in question consisted of nearly pure aniline, of toluidine, or of a mixture of the two. The method of manufacturing the different varieties of aniline oil is substantially the same, the composition of the product depending on that of the hydrocarbon employed. The details of the method of manufacture are, of course, subject to variation, but the following is an outline of the method pursued in a well-known aniline works: Crude coal-tar

naphtha is redistilled to a temperature of 170°. The product of the distillation, called "once-run naphtha," is treated with strong sulphuric acid (sp. gr. 1.845), which removes the bases, hydrocarbons of the olefine and acetylene series, and some of the higher homologues of benzene. A subsequent treatment with milk of lime or sodium hydroxide eliminates the phenols and other substances of an acid character. The purified oil is washed with water and redistilled to obtain "50/90 benzol," and this when fractionated with the aid of a dephlegmating column at once yields 99% benzol, toluol, and solvent naphtha. Solvent naphtha is now generally further treated for the isolation of xylene, but the benzol and toluol are directly converted into the nitro-compounds by placing them in a vessel surrounded with cold water, and gradually running in a cold, previously made mixture, of 150% by weight of nitric acid of 1.4 sp. gr. with 200% of concentrated sulphuric acid. When the reaction is complete the mixture is allowed to stand, and the lower layer of acid is tapped off and concentrated again for repeated use. The nitrobenzol is washed with sodium hydroxide, and then treated with open steam to drive off unchanged benzol and "light stuff." The nitrobenzol (or nitrotoluol obtained in a precisely similar manner) is then placed in a still with hydrochloric acid, and borings or filings of grey cast iron added gradually. High pressure steam is blown in, and the nitrobenzol which distils over is separated from the condensed water, and returned to the still until the complete solubility of the distilled oil in hydrochloric acid shows that the reaction is complete. Milk of lime is then introduced, and the liberated aniline distilled off by the aid of steam. Aniline sinks to the bottom of the condensed water, but when toluidine is being made the oil floats on the surface. The condensed water contains from 2 to 3% of dissolved bases, and is converted into steam for the aniline stills. The iron is converted into a black paste, consisting chiefly of Fe_3O_4, which is sold for purifying gas. The aniline oil is distilled to separate water, etc. The addition of lime to liberate the aniline is not strictly necessary, and in many works it is omitted. The first reaction seems to be:

$$C_6H_5.NO_2 + Fe_3 + 6HCl = 3FeCl_2 + C_6H_5.NH_2 + 2H_2O.$$

The ferrous chloride formed also acts as a reducing agent, being converted into ferric chloride, which in presence of water gives ferric

oxide and aniline hydrochloride. The end-products are chiefly aniline, ferroso-ferric oxide, and a weak solution of ferrous chloride. The hydrochloric acid seems to act chiefly as a carrier, so that the general reaction may be represented by the equation: $4C_6H_5.NO_2 + 9Fe + 4H_2O = 3Fe_3O_4 + 4C_6H_5.NH_2$. Acetic acid was formerly employed in place of hydrochloric acid, but its use is now almost, if not entirely, obsolete. Too large an excess of iron, or its too rapid addition, may cause loss from a reproduction of benzene, whilst deficiency of both iron and acid favours the production of azo-benzene.

Composition and Analysis of Aniline Oils

There are 2 leading kinds of aniline oil now recognised in the market, namely: 1. Pure aniline oil; and aniline oil for red. Three kinds of toluidine are commercially available: (*a*), mixed toluidine, the normal mixture of ortho and para, with a little meta resulting from the reduction of the normal mixed nitrotoluol previously described and used principally for fuchsine manufacture, (*b*), pure ortho and (*c*) pure paratoluidine. The demand for xylidine for the manufacture of azo-reds has considerably influenced the character of commercial aniline, since the 50/90 benzol, which was commonly used for the manufacture of "aniline for red," formerly contained a notable quantity of xylene, which is now removed and converted separately. Since the employment of dephlegmating columns has become usual, benzene and toluene of almost constant b. p. have been manufactured. From the pure hydrocarbons the corresponding bases are prepared, whilst from the intermediate oil, containing about 25% of benzene and 75 of toluene, an aniline oil for red is manufactured, which contains about 25% of aniline, from 20 to 25 of *p*-toluidine, and 45 to 50% of *o*-toluidine.[1]

In addition to the foregoing leading qualities of aniline oil, products of very varying composition and degrees of purity have to be dealt with by the dye-manufacturer. Thus in making magenta fully one-fourth of the aniline distils off and is condensed. But this recovered aniline is found on rectification to have a considerably higher density than the original oil (1.015 to 1.009 against 1.0075), and to consist almost entirely of aniline and *o*-toluidine, whereas the original oil contained from 15 to 25% of *p*-toluidine. This is either

[1] The composition of aniline oil for red is often judged of by the consumer solely from the sp. gr., and he or the aniline-maker adjusts it accordingly by adding aniline or toluidine to the crude oil as the gravity may indicate.

employed for the manufacture of safranine or very red shades of blue, or crude *p*-toluidine is added to it in such proportion as to bring it approximately to the original composition. In the manufacture of magenta by the nitrobenzene process, the recovered aniline contains notable quantities of nitrobenzene, whilst from other processes methylated and ethylated anilines are obtained. *Recovered anilines* are deeper in colour and of greater body than unused oils, and often have a strong and somewhat characteristic odour. They are rarely met with outside the colour works in which they have their origin.

On the next page is a tabulated list of the more important or frequently occurring constituents of aniline oils[1] recovered from industrial operations. With the exception of aniline and its homologues, and the substituted anilines, very little is known respecting the effect of the substances formulated in the table on the colouring matters produced. For the most part, the objectionable impurities are got rid by fractionating the crude aniline oil.

The *analysis* of aniline oils is usually limited to observations of the colour, odour, and sp. gr.. supplemented by a careful fractional distillation and tests for water content of basic constituents, solubility in HCl, nitrobenzene, hydrocarbons, etc.

The *sp. gr.* of aniline oil is a valuable indication of its composition. The observation must be made by the plummet or sp. gr. bottle at exactly 15°, and the result referred to water at the same temperature taken as unity.

The following figures represent the densities as thus observed:

	Sp. gr. at 15°.
Pure aniline,	1.0268.
Aniline oil for red,	1.0075 to 1.0012.
o-Toluidine,	1.0037.
Mixture of equal parts of *o*- and *p*-toluidine,	0.9975.
p-Toluidine (solid),	1.046.

The odour of pure aniline is very different from that of the toluidines. The presence of toluidine in aniline is indicated by the density of the sample, its diminished solubility in dilute alcohol, and by the results of the fractional distillation. In addition to these characters, the following tests are sometimes of service:

[1] Hell and Rockenbach (*Ber.*, 1889, **22**, 505) have investigated some other non-basic constituents of aniline and toluidine tailings.

PROPERTIES OF ANILINE, DERIVATIVES, ISOMERS, ETC.

Name	Formula	M. p.	B. p.	Remarks
Aniline	$C_6H_5NH_2$	−8°	183.7°	....
Toluidine *o*-; 1:2	$C_6H_4(CH_3).NH_2$	below − 20°	199°	....
Toluidine *m*-; 1:3	$C_6H_4(CH_3).NH_2$	below − 13°	197°	....
Toluidine *p*-; 1:4	$C_6H_4(CH_3).NH_2$	45°	198°	....
Xylidine (several isomers)	$C_6H_3(CH_3)_2.NH_2$	....	212–226°	....
Cumidine (several isomers, chiefly pseudo-cumidine)	$C_6H_2(CH_3)_3.NH_2$	63°	235°	....
Methyl-aniline	$C_6H_5NH(CH_3)$	....	192°	....
Dimethyl-aniline	$C_6H_5.N(CH_3)_2$	0.5°	192°	....
Ethyl-aniline	$C_6H_5.NH(C_2H_5)$	....	204°	....
Monomethyl-ortho-toluidine	$C_6H_4\text{-}CH_3\text{-}NH\text{-}CH_3$	....	207–208°	Sp. gr. at 15°—0.973.
Properties of Aniline, Derivitives, Isomer	$C_6H_4\text{-}CH_3\text{-}N\text{-}(CH_3)_2$	....	183°	....
Monoethyl-ortho-toluidine	$C_6H_4\text{-}CH_3\text{-}NH\text{-}C_2H_5$	....	213–214°	Sp. gr. at 15.5°—0.9534.
Diethyl-ortho-toluidine	$C_6H_4\text{-}CH_3\text{-}N\text{-}(C_2H_5)_2$	....	208–209°	....
Diphenylamine	$C_6H_5.NH(C_6H_5)$	54°	302°	....
Acetanilide	$C_6H_5.NH(C_2H_3O)$	112°	295°	....
Acetotoluide *o*-	$C_6H_4(CH_3).NH(C_2H_3O)$	65–66°	302–304°	Produced by action of heat on toluidine acetate.
Acetotoluide *p*-	$C_6H_4(CH_3).NH(C_2H_3O)$	147°	300–307°	Produced by action of heat on toluidine acetate.
Nitranilines	$C_6H_4(NO_2).NH_2$.	....	....	From imperfect reduction of dinitrobenzene.
Paraniline	$C_{12}H_{14}N_2$	192°	330°	....
Xenylamine	$C_{12}H_9.NH_2$	45°	322°	....
Phenylene-diamine (*para*-).	$C_6H_4:(NH_2)_2$	63°	287°	Reduction of dinitrobenzene.
Toluylene-diamine (*para*-).	$C_6H_3(CH_3):(NH_2)_2$	99°	283–285°	....
Azobenzene	$C_6H_5.N_2.C_6H_5$	65°	293°	Imperfect reduction of nitrobenzene.
Nitrobenzene	$C_6H_5.(NO_2)$	3°	210°	....
Dinitrobenzenes *o*-	$C_6H_4(NO_2)_2$	118°	....	Monoclinic tables.
Dinitrobenzenes *m*-	$C_6H_4(NO_2)_2$	90°	....	Long needles or thin rhombic tables.
Dinitrobenzenes *p*-	$C_6H_4(NO_2)_2$	172°	....	Monoclinic needles.
Nitrotoluenes *o*-	$C_6H_4(CH_3)(NO_2)$	below − 20°	223°	Sp. gr. 1.163 at 23.5°.
Nitrotoluenes *m*-	$C_6H_4(CH_3)(NO_2)$	16°	230°	Sp. gr. 1.168 at 22°.
Nitrotoluenes *p*-	$C_6H_4(CH_3)(NO_2)$	54°	238°	
Benzene	C_6H_6	5.5°	80.5°	....
Toluene	$C_6H_5(CH_3)$	below − 20°	111°	....
Aminothiophene	$C_4H_3S.NH_2$	....	... .	....
Paraffins	C_2H_{2n+2}	....	... . ..	Especially in aniline oils derived from cannel-tar benzols.

Pure aniline affords no rosaniline on treatment with oxidising agents, but if toluidine be present, magenta is readily formed. The test is best made by mixing 5 c.c. of the sample of aniline with an equal volume of a concentrated solution of arsenic acid, containing about 75% of As_2O_5 and having a density of 2.04. The mixture, contained in a small flask or long test-tube, is immersed in a paraffin-bath heated to 180°. The mixture rapidly changes in colour, and swells considerably. When the action is complete, the contents of the tube acquire a metallic bronze appearance and no longer intumesce. The product is treated with boiling water, when, if the sample contained toluidine, arsenate of rosaniline dissolves and communicates an intense crimson colour to the liquid. Neither pure aniline nor toluidine alone gives this reaction.

If a sample of commercial aniline be mixed with some solid magenta and a few drops of glacial acetic acid, and the whole heated to 180°, as described above, ammonia is abundantly evolved, and in a short time the mixture becomes intensely blue from the formation of triphenyl-rosaniline. With pure aniline the blue is very pure in shade, but when toluidine or xylidine is treated in a similar manner the product is intensely purple, and a mixture of the bases gives proportionate intermediate shades of colour. If a little of the "melt" be withdrawn from the tube, diluted considerably with alcohol, a few drops of acetic acid added, and then streaked on white filter-paper by means of a glass rod, the purple tint is readily observed, especially if the paper be held up before a gas-flame.

A valuable indication of the general composition of an aniline oil is obtained by submitting the sample to fractional distillation, and noting the proportions of distillate obtained at various temperatures. The distillate may be measured after each rise of 5° in the b. p. of the sample, or the temperature may be observed when each consecutive 5 or 10% fraction has passed over. The latter is the plan now commonly adopted, 100 c.c. of the sample being employed, and the arrangement of the apparatus being exactly the same as in the fractional distillation of benzols.

The heat is applied cautiously at first, in order to dissipate any water. When this is effected, which will be known by the rapid rise of the thermometer, the heat is so regulated that the distillate shall fall in distinct drops, about 60 per minute. With each increase of 10 c.c. in the volume of the distillate the temperature indicated by

the thermometer is observed and recorded, the process being continued till 90 or 95 c.c. have passed over.

A very simple test for aniline oils was devised and communicated to Allen by B. Nickels, who found it to give useful results, and to indicate differences between samples not readily distinguishable by the ordinary fractional distillation process. The test is based on the greater solubility in dilute alcohol of aniline as compared with toluidine and xylidine, and is thus performed: 5 c.c. volume of the sample is taken with a pipette and diluted to 40 c.c. with methyl alcohol. Distilled water is then gradually added from a burette, with constant shaking, till a permanent turbidity is produced, when the volume of water employed is noted. Treated in this way, a sample of very pure aniline required 126 c.c. of water to produce permanent turbidity. The following figures, obtained by Nickels in 1881, show the results yielded by three typical specimens of commercial aniline as then manufactured:

	A Pure aniline	B Heavy aniline	C Toluidine
Colour	Pale amber	Amber	Deep brown
Sp. gr. at 15.5°	1.025	1.011	1.002
Water required for precipitation	106.4 c.c.	73.7 c.c.	63.2 c.c.
10% distilled over at	183¼°	189°	195°
20% distilled over at	183½	189¾	195¾
30% distilled over at	183¾	190	196
40% distilled over at	184	191	196½
50% distilled over at	184¼	191¼	197
60% distilled over at	184	192¼	197½
70% distilled over at	184	193	198
80% distilled over at	184	194⅓	198½
90% distilled over at	184½	197	199¾
95% distilled over at	184¾	201	....

Sample A was a fair commercial specimen of the quality known as "pure aniline," and actually contained some 95% of real aniline. An article of this high purity is required for the manufacture of aniline blue, triphenyl-rosaniline, any notable admixture of toluidine resulting in a product dyeing with reddish tinge.[1]

The quality known as "heavy aniline," exemplified by B, is a fair sample of aniline oil for red. This class of aniline is produced from

[1] In good samples the b. p. is fairly constant, differing by 1 or 2 only. Inequalities or jumps in the b. p., especially at the beginning and end of the distillation, indicate badly-made samples or mixtures.

benzols containing a considerable proportion of toluene, and the aniline oil itself is a mixture of aniline and toluidines. Good samples of aniline oil for red contain from 35 to 42% of real aniline, 35 to 50% of *o*-toluidine, and 14 to 24% of *p*-toluidine.

R. J. Friswell thinks 100 c:c. an undesirably small quantity for fractional distillation. He prefers to operate on 250 c.c. which he distils in a flask with a side-tubulure, and he recommends an observation of the temperature at which the last drop disappears from the bottom of the flask.[1] A naked flame is used, and a few fragments of platinum wire or fire-brick added to the contents of the flask. The following figures were obtained by Friswell (Thorpe's *Dict. Applied Chem.*, 1, 165) by the examination of commercially pure aniline.

	No. 1	No. 2	No. 3
Sp. gr. at 15°	1.02710	1.02684	1.02690
10% over at	184.7°	184.6°	184.6°
20% over at	184.7	184.8	184.6
30% over at	184.7	184.8	184.7
40% over at	184.7	184.8	184.7
50% over at	184.8	184.8	184.8
60% over at	184.9	184.8	184.8
70% over at	185.0	184.8	184.9
80% over at	185.1	184.8	184.9
90% over at	185.1	184.8	185.0
Dry at	186.7	186.8	....

Any *water* present in aniline oil will be found in the very first portions (first fraction of 10%) whenever the sample is submitted to distillation. It takes the form of globules, which are not miscible with the next fraction of the distillate nor with petroleum spirit. Water may exist in aniline in any proportion from a trace up to 3 or 4%, but a good commercial rectified specimen should not contain more than 0.5%. Aniline is readily soluble in a strong aqueous solution of aniline hydrochloride. A solution of the kind, of 1.08 sp. gr., was stated by Watson Smith to be sometimes sold as ailine oil, which in colour and taste it closely resembles. Such a fraud would be at once detected on distillation.

Benzene, toluene, and other **hydrocarbons** will separate when the first fraction of 10% (10 c.c.) is treated with an equal volume or slight

[1] Note by Amer. Editors. It is better to distil to a point where the thermometer ceases to rise. With high-boiling compounds such as these the flask often becomes superheated on the sides and the condensation drops are likely to crack on meeting these hot surfaces. This occurs when from 95 to 98 % has distilled over, and with very crude samples, sometimes when less has passed over.

excess of hydrochloric acid, and water added to 100 or 150 c.c. They assume the form of oily globules which float even on diluting the liquid. The best samples of pure aniline show only a slight opalescence when thus treated, but the smell of the "light stuff" is always perceptible. In recovered anilines these impurities exist to a notable extent, since they survive the reactions by which the bases are consumed. Aniline for red usually contains somewhat more hydrocarbons than pure aniline.

Nitrobenzene and **nitrotoluene** may be recognised, even when mere traces are present, by the milky appearance of the liquid produced by saturating 10 c.c. of the original sample of oil with hydrochloric acid. On diluting the liquid with water, and leaving it at rest for some hours, any considerable quantity of nitrobenzene will collect at the bottom in the form of oily globules, which, after separating the acid liquid, may be identified by the smell and other characters. Still smaller quantities of nitrobenzene may be recognised if the "tailings" be operated upon instead of the original sample. Good commercial aniline oil, when placed in a bottle and shaken should, show a white foam. Even traces of nitro compounds cause the foam to be yellow, and such a product is almost unsalable. Nitrobenzene occurs more frequently in magenta-aniline and toluidine than in the oils of lower b. p. The content of nitrobenzene in aniline may be accurately estimated by steam distilling an acid solution of a known weight of "oil" and titrating the nitrobenzene in the distillate with titanous chloride according to the procedure of Knecht and Hibberd. This is a very accurate method. In a similar fashion nitro compounds present in mixed toluidines, toluidines, xylidines etc. may be estimated.[1]

Acetanilide and **acetotoluide** were impurities characteristic of aniline prepared by the reduction of nitrobenzene with acetic acid and iron, but are now rarely met with in aniline oils. In any case, they would become concentrated in the "tailings," together with phenylene-diamine, azobenzene, paraniline, xylylamine, etc.

Aniline tailings is the name applied to the least volatile portion of aniline oils. They contain little or no aniline; some toluidine, xylidine, and cumidine; nitrobenzene and its homologues; and some or all of the by-products which boil above 200°.

[1] Amer. Editors.

The content[1] of amino-compound or compounds in commercial aniline, aniline oils, toluidines etc. may be conveniently and accurately estimated by two general methods: The diazotisation method which may be applied by dissolving a small amount, about 2 to 3 grm. of the oil (in excess hydrochloric acid), about 25 to 30 c.c. concentrated HCl, with about 200 c.c. of water. The solution is cooled to 0° and made up to about 800 c.c. with ice water. The solution is then titrated with N/2 sodium nitrite solution, starch iodide paper being used as indicator. The diazobenzene chloride is very unstable and the titration must be conducted as near zero as possible. Diazobenzene chloride solution is much used in the analysis of dye intermediates.

The second and more accurate method is that based on the use of N/10 $KBrO_3.KBr$ solution. Weigh about 3.0 grm. of aniline carefully from a large pipette into a 1000° c.c. volumetric flask containing about 900 c.c. of distilled water and about 50 c.c. of concentrated hydrochloric acid. Make up to the mark. Transfer 100 c.c. aliquot parts, add 10 c.c. concentrated hydrochloric acid, cool the solution to 15° or below and titrate slowly with N/10 $KBrO_3.KBr$ solution finishing the titration, drop by drop, testing with starch-iodide papers. A 2 minute end-point is required, avoiding deep blue coloration. Use same end point as that used in the standardisation. Aniline forms a tribromo compound.

This method is applicable, but the solution should be standardised against the particular amino compound being estimated.

The composition and special methods of examination of commercial *toluidine* are described on page 550 *et seq.*

Anilides

The anilides are derivatives of aniline in which one or both of the hydrogen atoms of the amino-group are replaced by acid-radicals. The homologues of aniline yield similar derivatives (*e. g.*, aceto-toluide. The most important and typical member of the class is acetanilide or phenylacetamide:

$$C_6H_5.NH(C_2H_3O); \text{ or } C_2H_3O.NH(C_6H_5).$$

A number of derivatives of acetanilide have been prepared, and certain of them have found some employment as analgaesics and antipyretics, as for instance:

[1] Amer. Editors.

Acetanilide. Phenylacetamide. Antifebrin.	$C_6H_5.NH(C_2H_3O)$.
Bromacetanilide. Antiseptin. Brominated antifebrin.	$C_6H_4Br.NH(C_2H_3O)$.
Methylacetanilide. Exalgin. Methylated antifebrin.	$C_6H_5.N(CH_3)(C_2H_3O)$.
Aceto-amidophenol. Hydroxy-antifebrin.	$C_6H_4(OH).NH(C_2H_3O)$.
Aceto-anisidine. Methacetin. Methoxy-antifebrin.	$C_6H_4(O.CH_3).NH(C_2H_3O)$.
Acet-phenetidine. Phenacetin. Ethoxy-antifebrin.	$C_6H_4(O.C_2H_5).NH(C_2H_3O)$.
Amido-phenacetin. Phenocoll.	$C_6H_4(O.C_2H_5).NH(C_2H_2O.NH_2)$

Most of these substances are described in the following pages. The relationship of antifebrin to hypnone, hydracetin (pyrodine), and phenyl-urethane, is shown by the following formulæ:

Acetophenone. Hypnone.	$C_6H_5.(CO.CH_3)$.
Acetanilide. Antifebrin (see below).	$C_6H_5.NH.(CO.CH_3)$.
Acet-phenylhydrazine. Hydracetin.	$C_6H_5.NH.NH.(CO.CH_3)$.
Lævulinyl-phenylhydrazine. Antithermin.	$C_6H_5.NH.NH.(C_5H_7O_2)$.
Phenyl-urethane. Euphorin.	$C_6H_5.NH.(CO.O.C_2H_5)$.

Acetanilide. Phenylacetamide. $C_6H_5.NH(C_2H_3O)$

This substance was originally obtained by the action of acetyl chloride on aniline. It is more conveniently prepared by boiling aniline with glacial acetic acid for many hours under an inverted condenser, until the product solidifies on cooling. The mass is then melted and poured into water, to remove unconverted aniline and acetic acid. It may be purified by distillation and crystallisation from alcohol, benzene, or hot water, from which it separates in colourless unctuous laminæ, resembling boric acid, soluble in about 190 parts of cold or 18 of boiling water. Acetanilide is odourless, but produces a slight burning sensation on the tongue. It occurs commercially as a crystalline powder or scales. It melts at 113°, and distils unchanged at 295°. Acetanilide dissolves in 3.5 parts of alcohol, and is very soluble in ether, chloroform, and benzene, yielding neutral solutions.

Acetanilide is a weak base. The *hydrochloride* is obtained by passing hydrochloric acid gas through a solution of acetanilide in acetone. It forms needles which are decomposed into their constituents by water, and gradually converted into acetic acid and aniline hydrochloride on exposure to moist air.

Acetanilide dissolves in strong sulphuric acid, without change of colour. On treating the solution with nitric acid, the acetanilide is converted chiefly into *para*-nitroacetanilide, some of the *ortho*-compound and, in presence of a large excess of sulphuric acid, a little of the *meta*-compound being also formed. Nitrous acid, passed into its acetic acid solution, converts acetanilide into an unstable nitrosamine, $C_6H_5.N(C_3H_3O)(NO)$. When heated with zinc chloride to about 250°, acetanilide yields *flavaniline*, $C_{16}H_{14}N_2,HCl$. Treated in alcoholic solution with sodium ethylate, acetanilide yields a sodium derivative, $C_6H_5.NNaC_2H_3O$, but when this is boiled with water it splits into aniline and sodium acetate. Acetanilide behaves like aniline on treatment with alkali hydroxide and chloroform (page 538), and the formation of the disagreeably smelling isonitrile is a delicate reaction for its presence.

Acetanilide behaves like aniline when treated with phenol and a solution of bleaching powder (page 539).

When treated with a solution of potassium chlorate in strong sulphuric acid, acetanilide gives a red coloration, changed to yellow on dilution. With a crystal of a nitrite and a drop of concentrated hydrochloric acid it produces a yellow colour, changing on heating to green and blue; and, on evaporating the liquid to dryness, an orange residue is obtained, changed to red on adding ammonia (Vitali).

When acetanilide is heated gently with mercurous nitrate, a substance is produced which dissolves in alcohol with green colour (Yvon). If a few centigrams of acetanilide be gently heated with 2 or 3 drops of a solution of mercurous nitrate, and when solution has been effected 2 or 3 drops of sulphuric acid added, a blood-red coloration will be produced (Cella and Arzeno). The same reaction is produced by phenol, resorcinol, thymol, and salicylic, gallic, and tannic acids, but not by benzoic acid.

Acetanilide gives no colour-reactions with ferric chloride, nitrites in very dilute solutions, or potassium dichromate in aqueous solution. These reactions distinguish it from antipyrine and kairine.

Various other colour reactions of acetanilide have been described. The tests given in the *United States Pharmacopœia* are included in those given herewith. As a rule, the most satisfactory method for its positive identification is to heat the substance with alcoholic potassium hydroxide, dilute with water, and shake with ether. The ethereal layer is examined for aniline, while the aqueous liquid is tested for an acetate.

To detect acetanilide in urine, Vulpius boils the liquid with hydrochloric acid, cools, extracts with ether, and tests the ethereal solution with phenol and bleaching powder solution.

E. Ritsert (*Pharm. Zeit.*, 1890, **35**, 306) gives the following tests for the purity of commercial acetanilide: The sample should leave no ash on ignition, and after drying for 2 hours at 105°, should melt at 114°. A higher or lower m. p. indicates the presence of acetotoluides. 0.1 grm. dissolves in 1 c.c. of strong hydrochloric acid to a clear solution, which, after a few minutes, precipitates acetanilide hydrochloride (methyl-acetanilide does not yield a similar reaction). No change should be produced on adding a drop of nitric acid, which, after a time, produces a yellow or brown coloration if phenacetin or methacetin be present. If 0.1 grm. be boiled in portions in 2 c.c. of strong hydrochloric acid, the solution cooled, and a drop or two of chlorine water added, a fine blue coloration is produced. The aqueous solution of acetanilide should be free from acid reaction (indicating acetic acid). On boiling it and adding ferric chloride, a deep reddish-brown colour should be produced, destroyed by a mineral acid. If a drop of dilute solution of potassium permanganate (1:1000) be added to a boiling aqueous solution of 1 grm. of acetanilide in 30 c.c. of water, the pink coloration at first produced should persist at least 5 minutes, and should not change to yellow on again boiling. Precipitation at this stage indicates the presence of free aniline, resinous products, acetotoluides, or other impurities.

In the current *British Pharmacopœia*, (of 1914) acetanilide is described as melting at 113°, and dissolving in sulphuric acid without coloration. A cold saturated aqueous solution is not coloured on adding ferric chloride. Bromide solution gives a yellow-white precipitate. In the *German Pharmacopœia*, the direction is to add ferric chloride to a cold saturated solution, thus avoiding the dissociation and formation of acetic acid liable to occur on

boiling. According to the *German Pharmacopœia*, on heating with alkali hydroxide solution, acetanilide gives off an aromatic vapour, which, after addition of a drop of chloroform and renewed application of heat, is changed to the disagreeable smell of the iso-nitrile. Further, 0.1 grm. of acetanilide should yield a clear solution adding to the liquid 2 c.c. of carbolic acid, a cloudy red coloration should be produced by solution of bleaching powder, changed to a permanent indigo blue (indophenol) on adding excess of ammonia.

Acetanilide has powerful antipyretic properties, and has received an extensive application in medicine under the name of "anti-febrin,"[1] though dangerous symptoms are sometimes produced by it (*Pharm. Jour.*, [iii], **20**, 1059). The dose is from 3 to 10 grains.

According to Salzer, commercial antifebrin is liable to contain unchanged aniline, which may be detected by dissolving the sample in cold hydrochloric acid, and pouring on the liquid a solution of bleaching powder. Pure acetanilide yields a white precipitate, which dissolves on shaking the liquid, but after a time colourless silky needles separate. In presence of aniline the well-known violet colouration is produced.

Acetanilide has been used as an adulterant of antipyrine. The m. p. of the pure substances are nearly identical, but a mixture of equal proportions of the two melts at 45°.

ESTIMATION OF ACETANILIDE AND PHENACETIN IN ADMIXTURE

Phenacetin.—W. O. Emery (*J. Ind. Eng. Chem.*, 1914, **6**, 665) gives the following procedure: Into a small (50 c.c.) lipped Erlenmeyer flask introduce 0.2 grm. of the phenacetin-acetanilide mixture, add 2 c.c. of glacial acetic acid, heat gently over wire gauze to complete solution, then dilute with 40 c.c. of water previously warmed

[1] When administered to rabbits, acetanilide is oxidised to para-aminophenol, $C_6H_4(OH).NH_2$, with complete elimination of the acetyl group. In dogs there is a small formation of para-aminophenol, but the chief change consists in a simultaneous oxidation of the aniline residue to ortho-aminophenol, of the acetyl group to carboxyl, and in the formation of carbonyl-ortho-hydroxyamidophenol, $C_6H_3(OH)\left\{\begin{matrix}NH\\O\end{matrix}\right\}CO$, the anhydride of which is excreted in the urine as a sulphate. In both the rabbit and the dog the amido-phenols are also eliminated as sulphates. In man, the acetyl group is not wholly oxidised, the urine containing the sulphate of aceto-paraminophenol. In all cases there is an oxidation of one of the hydrogen atoms of the benzenenucleus to hydroxyl, whilst the proportion of ethereal sulphates is increased, the urine is red from excess of bilirubin, reduces alkaline cupric solution, and is strongly lævorotatory, the optically active substance probably being the above-mentioned sulphate (Gressly and Nencki, *Monatsh*, 1890, **11**, 253).

to 70°. Transfer the clear aqueous liquid, by pouring and careful washing of the flask with two 10 c.c. portions of warm (40°) water, into a glass-stoppered, graduated 100 c.c. flask, into which have been previously run from a burette 25 c.c. of standard iodine, of a strength slightly above N/5 and warmed to 40°. Rotate the resulting menstruum to uniformity, the flask being closed meanwhile, then add 3 c.c. of concentrated hydrochloric acid, close the flask again and continue rotation until copious crystallisation is apparent, and then set the product aside to cool. If the ratio of phenacetin to acetanilide is equal to or greater than 1, crystalline scales will form almost immediately on adding the acid. As the proportion of acetanilide increases, however, the periodide is not only more inclined to maintain the liquid state, with the result that crystallisation becomes proportionately slower, but its separation from the menstruum itself is in a measure retarded. In such cases, gentle agitation of the liquid or rotation of the flask in water warmed to 40° or less tends to promote the formation of crystals. When the contents of the flask have assumed the temperature of the room, fill up with water to within 2 to 3 c.c. of the mark, rotate to uniformity and leave over night. Fill to the mark with water, mix thoroughly, then, after standing 30 minutes, withdraw a 50 c.c. aliquot part of clear liquid by passing through a small (5.5 cm.) dry, closely fitted filter into a graduated 50 c.c. flask; the first 15 c.c. of the first runnings should be rejected, being received in any convenient container for eventual use later, along with additional filtrate, for the recovery of acetanilide. Transfer the 50 c.c. aliquot part by pouring and washing to a 200 c.c. Erlenmeyer flask and titrate with N/10 sodium thiosulphate solution.

If the composition is considered of the insoluble addition product, $(C_2H_5O.C_6H_4NH.COCH_3)_2.HI.I_4$ formed in the foregoing separation, it will be noted that, for every molecule of phenacetin involved, 2 atoms of iodine are required; hence from a titrimetric standpoint, 1 atom of iodine is equivalent to ½ mol. of phenacetin. If, therefore, the quantity of iodine expended in the formation of insoluble periodide is ascertained as the result of such titration, the quantity of phenacetin thereby involved is readily calculated from the expression,

$$\text{phenacetin} = I\ (0.008890 \times N)$$

in which 0.008890 represents the quantity of phenacetin in 1 c.c. of an N/10 solution of this substance, N the normality of standard

thiosulphate employed, while I represents the number of c.c. of such combination with phenacetin isolated as periodide.

The gravimetric estimation of phenacetin may, if desired, be effected substantially as follows: In the operation of filtering off the periodide, the latter is collected on the filter and washed with 10 to 15 c.c. of standard iodine solution, preferably by suction, then transferred, together with the filter (likewise any particles of precipitate which may remain in the graduated flask) to a separating funnel, using for the purpose not more than 50 c.c. of water. After discharging both free and added iodine with a few small crystals of sodium sulphite, the liquid is extracted with three 50 c.c. portions of chloroform, each portion being subsequently washed in a second separating funnel with 5 c.c. of water. After washing and clearing, the solvent is passed through a small (5.5 cm.) dry filter into a 200 c.c. Erlenmeyer flask, most of the chloroform removed by distillation, and the residual 5 to 10 c.c. are transferred by pouring and washing with fresh solvent into a small tared beaker or crystallising dish. The solution is evaporated to dryness on the steam-bath, and the residue cooled and weighed.

Acetanilide.—Should the combined weight of the phenacetin-acetanilide mixture be known, that of the latter constituent can be determined by difference, or, if necessary, estimated directly from a second aliquot part of the filtrate from the phenacetin-periodide.

To this end, transfer to a separating funnel by means of a pipette 25 to 30 c.c. of the clear liquid, decolorise with sufficient solid sodium sulphite, add solid sodium bicarbonate in slight excess, follow with 1 to 2 drops of acetic anhydride, then extract with three 60 c.c. portions of chloroform, passing the solvent when cleared through a small, dry filter into a 200 c.c. Erlenmeyer flask; the chloroform is distilled off by the aid of gentle heat until the volume is about 20 c.c. Now add 10 c.c. of dilute sulphuric acid (1 c.c. of concentrated acid to 10 c.c. of water) and digest the product on the steam-bath until the aqueous residue has been reduced to one-half, add 20 c.c. of water and continue the digestion 1 hour, add a second 20 c.c. portion of water and 10 c.c. of concentrated hydrochloric acid, then titrate very slowly, drop by drop, with standard potassium bromide-bromate (1 c.c. of which is equivalent to 5 to 10 mg. of acetanilide), until a faint yellow coloration persists.[1] While adding this

[1] Starch-KI paper is a more accurate indicator for this end-point. Amer. Editors.

reagent, the flask should be rotated sufficiently to agglomerate the precipitated tribromaniline and thus clarify the supernatant liquid. The number of c.c. of standard bromide solution required to complete the precipitation, multiplied by the value of 1 c.c. in terms of acetanilide, will give the quantity of this substance present in the aliquot part taken.

Of the 3 isomeric *aceto-toluides*, only the meta compound possesses antipyretic properties.

Diacetanilide, $C_6H_5N:(CH_3CO)_2$, is a crystalline substance melting at 111°. It is purified by crystallisation from benzine.

Formanilide, phenyl formamide, $C_6H_5.NH(HCO)$. Colourless crystals, m. p. 46°, soluble in water, alcohol and glycerin. This substance is prepared by heating aniline and formic acid in a way similar to the preparation of acetanilide. It is an antipyretic analgaesic and local anaesthetic.

Gallanilide, gallanol, $C_6H_5NH.CO.C_6H_2(OH)_3$, forms colourless crystals of bitter taste, m. p. 205°. Hot water, alcohol and ether are solvents. It is used as an astringent for wounds.

Para-brom-acetanilide, $C_6H_4Br.NH(CO.CH_3)$, has been introduced as a remedy under the name of "antisepsin." It forms small pearly prisms, melting at 164.5°, and devoid of taste or smell. It is soluble with difficulty in cold, but readily in hot water, as also in alcohol and ether.

Acet-methylanilide or methylacetanilide, $C_6H_5.N(CH_3)(C_2H_3O)$, is prepared by warming together methylaniline and acetyl chloride. The product is boiled with water, when the new substance crystallises on cooling. Methylacetanilide has been introduced as an anti-rheumatic and analgaesic under the name of "exalgin." In doses of 0.5 to 4 grains its effects are said to be very satisfactory. Exalgin forms fine needles or large white tablets (compare "Acetanilide"). It melts at 100–101°, boils without decomposition between 240 and 250°, and is slightly soluble in cold, but more so in boiling water, and very soluble in water containing a little alcohol. It is saponified with difficulty by alkali hydroxide, but completely by concentrated hydrochloric acid, with formation of acetic acid and methylaniline.

Acetanilide and methylacetanilide may, according to V. Zertio (*L'Union Pharm.*, 1910, **51**, 255), be distinguished by the following reactions: About 0.05 grm. is treated with 10 drops of hydrochloric

acid and boiled for 2 minutes. The liquid is then cooled, 5 more drops of hydrochloric acid are added, then 1 drop of 1% sodium nitrite solution; after allowing reaction to take place for 10 minutes, 1 c.c. of phenol is added and then, gradually, enough strong sulphuric acid to give a homogeneous mixture. Of this, 0.5 c.c. is treated with sufficient sodium hydroxide solution, sp. gr. 1.332, to give a clear solution. In the case of exalgin a blue colour will be obtained and with actanilide a yellow tint. Mixtures will naturally vary from yellowish to green. The various details of the test must be rigorously observed.

Methyl Acetanilide

Hirschsohn states that methylacetanilide may be distinguished from acetanilide and phenacetin by treating 1 grm. with 2 c.c. of chloroform, which dissolves the exalgin only. A chloroform solution of exalgin remains clear on adding 10 volumes of petroleum spirit, whereas the solutions of antifebrin and phenacetin become turbid. 20% of acetanilide, or 10 of phenacetin, may be detected in exalgin by these reactions. An aqueous solution of antifebrin gives a bromine derivative on adding bromine water, thus differing from exalgin and phenacetin.[1]

Benzanilide, $C_6H_5.NH(CO.C_6H_5)$, is obtained by the action of benzoyl chloride on aniline, or by boiling together equivalent quantities of benzoic acid and aniline. It forms a white, crystalline powder, m. p. 160–161° and volatile without decomposition. It is almost insoluble in water, but dissolves in 58 parts of cold, or 7 of boiling alcohol, crystallising, on cooling, in nacreous plates. It is difficultly soluble in ether. Benzanilide is not attacked by aqueous alkalies or acids, but is saponified by fusion with potassium hydroxide. It has been found valuable as an antipyretic for children, in doses of 2 to 8 grains, and is said not to produce objectionable secondary effects.

Phenyl-urethane, $C_6H_5.NH(CO.OC_2H_5)$.—This compound has acquired a practical interest owing to its introduction as a synthetic remedy under the name of "euphorin." It is produced by the reaction of aniline on ethyl-chlorocarbonate, and occurs as a

[1] Exalgin may also be distinguished from antifebrin, methacetin, and phenacetin by treating 0.1 grm. with 1 c.c. of concentrated hydrochloric acid. Phenacetin remains insoluble. Antifebrin dissolves, but separates again in crystals of the hydrochloride. Methacetin also dissolves, but is recognised by the reddish-brown coloration produced on adding 1 drop of nitric acid.

white crystalline powder, of a faintly aromatic odour and scarcely perceptible taste, which subsequently becomes acrid and clove-like. It melts at 49 to 51°, boils at 237°, and is only slightly soluble in cold water, but very freely soluble in alcohol, and sufficiently soluble in sherry and other alcoholic liquids to be conveniently given in solution in such menstrua. According to Sansoni, after administration of phenyl-urethane, the urine shows the para-aminophenol reaction either directly or after distillation with potassium carbonate. The proportion of urea is increased, but the urine is free from phenol, aniline, albumin, and sugar.

Substituted or Alkylated Anilines

These bases result from the replacement of one or both of the hydrogen atoms of the amino-group of aniline by alkyl or similar radicals.

The bases of this class are obtained by heating the hydrochloride or other salt of aniline (or its homologues) with the alcohol with which it is intended to react, or the halogen salt of this alcohol with free aniline.

The only substituted anilines which require special description are the following:

	Formula	Sp. gr.	B. p.
Methyl-aniline	$C_6H_5.NH(CH_3)$	0.976 at 15°	193.8
Dimethyl-aniline	$C_6H_5.N(CH_3)_2$	0.9553 at 15°	193.1
Ethyl-aniline	$C_6H_5.NH(C_2H_5)$	0.954 at 18°	204
Diethyl-aniline	$C_6H_5.N(C_2H_5)_2$	0.937 at 13°	216.5
Phenyl-aniline (Diphenylamine)	$C_6H_5.NH(C_6H_5)$	1 161	310
Diphenyl-aniline (Triphenylamine)	$C_6H_5.N(C_6H_5)_2$	. . .	....

Diphenylamine is a very weak base, and in triphenylamine the basic character is entirely lost.

Methyl-aniline. $C_6H_5.NH(CH_3)$.

This base is obtained by the action of iodide, nitrate, or chloride of methyl on aniline, or by heating methyl alcohol with aniline hydrochloride.[1] In all cases dimethyl-aniline is formed simultaneously,

[1] Pure methylaniline may be obtained by the reaction of methyl iodide on sodium acetanilide, $C_6H_5.NNa(C_2H_3O)$, and saponification of the resulting compound by alkali hydroxide.

and hence in the production of mono-methyl-aniline a portion of the aniline remains, in practice, unattacked.[1]

Methylaniline is a liquid boiling at 193.8°. It resembles aniline, but is lighter than water, and its odour is stronger and more aromatic. The *sulphate* is soluble in ether and uncrystallisable. A solution of bleaching powder first colours it violet and then brown.

Methylaniline-nitrosamine, $C_6H_5.N(CH_3)(NO)$, separates as a yellow oil on treating a cold solution of methylaniline hydrochloride with sodium nitrite, while any aniline and dimethylaniline are converted into soluble products. If the nitrosamine be extracted by ether, and treated with tin and hydrochloric acid, it is reduced to methylaniline, which may thus be obtained in a pure state. The nitrosamine is destitute of basic properties. It has an aromatic odour, and may be distilled in a current of steam, but not alone. When methylaniline-nitrosamine is warmed with phenol and sulphuric acid, the mixture diluted with water and saturated with alkali hydroxide, it yields the intense green-blue coloration produced by all nitrosamines (Liebermann's reaction). When heated with alcoholic hydrochloric acid it undergoes molecular transformation into paranitroso-methylaniline, $C_6H_4(NO).NH(CH_3)$, a substance crystallising in green-plates or steel-blue prisms, and otherwise resembling paranitroso-dimethylaniline.

Dimethyl-aniline. $C_6H_5.N(CH_3)_2$.

This important base is obtained by the action of excess of methyl iodide on aniline. On the large scale, methyl iodide was formerly employed, but was afterward replaced by the nitrate, and this again (owing to its explosive properties) was superseded by the very volatile methyl chloride. The product obtained in this way contained about 5% of monomethyl-aniline, but no other admixtures. Dimethyl-aniline is now always manufactured by heating together a mixture of aniline hydrochloride, aniline, and methyl alcohol.[2] The

[1] To separate this from its mono- and di-methyl derivatives, dilute sulphuric acid is added as long as aniline sulphate continues to separate. The sulphuric acid solution is separated from the solid aniline sulphate by pressure in a linen cloth, and the expressed liquid treated with sodium hydroxide. The substance which separates is dried and treated with acetyl chloride until no further rise of temperature is observed, when the product is poured into cold water. On cooling, methyl-acetanilide, $C_6H_5.N(CH_3)(C_2H_3O)$, separates in long needles, while dimethylaniline hydrochloride remains in solution. The former product is saponified by boiling with dilute hydrochloric acid, which converts it into acetic acid and methyl-aniline hydrochloride.

[2] The aniline must be free from toluidine and impurities insoluble in hydrochloric acid; and the methyl alcohol employed must be quite free from ethyl alcohol and acetone, the latter of which not only reduces the yield, but gives a product unsuitable for the preparation either of Methyl Violet, or Malachite Green, owing to the formation of a base of the formula $CH_2(C_6H_4.N(CH_3)_2)_2$. 93 parts of aniline are used, of which 18 are saturated with hydrochloric acid and 75 parts of methyl alcohol. The excess of methyl alcohol and

methyl alcohol employed must be quite free from ethyl alcohol and acetone, the latter of which not only reduces the yield, but gives a product unsuitable for the preparation either of Methyl Violet or Malachite Green, owing to the formation of a base of the formula: $CH_2(C_6H_4.N(CH_3)_2)_2$.

Dimethylaniline is a colourless oily liquid, solidifying at 0.5° and boiling at 193.1°. It has a sharp basic odour, and forms uncrystallisable salts. It unites with methyl iodide, with energy at the ordinary temperature, to form the iodide of trimethyl-phenylammonium, which breaks up again into its constituents on distillation, but by reaction with argentic oxide yields trimethyl-phenylammonium hydroxide, $Me_3PhN.OH$, a crystalline, very deliquescent, corrosive, and very bitter base.

With bleaching-powder solution, dimethylaniline merely gives a pale yellow coloration, a reaction by which any contamination by aniline or mono-methylaniline can be detected, as these bases give a violet colour with the same reagent. Mild oxidising agents, such as chloranile, carbon oxychloride, and cupric chloride, convert the methylaniline into Methyl Violet (Vol. 6). With acid chlorides and aldehydes, it yields complex compounds. Thus with benzaldehyde it gives tetra-methyl-paradiamino-triphenylmethane, and the corresponding hydroxide or carbinol, $C_6H_5.[N(CH_3)_2]_2.OH$, obtained from this by oxidation, is the base of Malachite or Benzaldehyde Green (Vol. 6). By reaction with diazobenzene chloride, dimethylaniline is converted into dimethyl-amino-azobenzene, $C_6H_5.N_2.C_6H_4.N(CH_3)_2$, or Butter Yellow; while with diazobenzene-sulphonic acid it yields Helianthin or Methyl Orange.

Paranitroso-dimethylaniline, $C_6H_4(NO).N(CH_3)_2$, is produced by the action of nitrite of sodium or nitrite of amyl on dimethylaniline.[1] It is manufactured on a large scale for the production of Methylene Blue, Indophenol, and Toluylene Red (Vol. 6). It crystallises in large green plates or tables, soluble in ether. By oxidation with potassium permanganate or ferricyanide, it is converted into

comparatively small quantity of hydrochloric acid tend to produce a purer oil. With more hydrochloric acid, the reaction takes place at a lower temperature, but there is a danger of forming toluidine. The mixture is heated at first to a temperature of 270°, at a pressure not exceeding 27 atmospheres. When the reaction is complete, in about 15 hours, the pressure decreases without the temperature being reduced (Schoop, *Chem. Zeit.*, 1887, 11, 253).

[1] Ten parts of dimethylaniline are dissolved in 50 of strong hydrochloric acid and 200 of water, and to the cold solution is gradually added a solution of 5.7 parts of sodium nitrite in 200 of water, when the hydrochloride of the nitroso-compound is obtained as a substance crystallising in yellow needles, from which the free base is obtained by treatment with potassium carbonate and solution in ether.

p-nitro-dimethylaniline, $C_6H_4(NO_2).N(CH_3)_2$, which forms long, sulphur-yellow needles, melting at 162–163°. When boiled with alkali hydroxide, nitroso-dimethylaniline is completely split up into dimethylamine, $H.N(CH_3)_2$ (which may, by this reaction, readily be obtained pure), and nitrosophenol or quinonoxime, $C_6H_4O(NOH)$.

Commercial dimethylaniline seldom contains much aniline and methylaniline. Very pure materials must be used in its production, and since its principle use is in the manufacture of Methyl Violet and Malachite Greens and the presence of aniline and methyl-aniline reduces the yield of dystuff and alters the shade, the presence of these compounds in more than 1–2% is of unusual occurance. Good commercial samples will boil over a very narrow range and show a purity in excess of 98%. Dimethyl-toluidine, $C_6H_4(CH_3).N(CH_3)_2$, and higher homologues are rarely met with. Dimethylaniline of commerce usually boils between 192 and 197°; the smaller the range in the b. p. the better the sample. Recent determinations on a very pure sample show a b. p. of 193.1° and a sp. gr. of .9621 $\left(\frac{15°}{15°}\right)$.

O. de Coninck (*Comptes Rend.*, 1900, **131**, 945–946) gives a summary of the reactions of substituted anilines.

The following reactions are given by alcoholic solutions of the bases with solutions of salts of copper, nickel, and cobalt:

	Methylaniline	Dimethylaniline	Ethylaniline	Diethylaniline
Dilute copper chloride.	Bluish opalescence, darkening gradually. In time, slight bluish-white precipitate. Fleeting white fluorescence.	Bluish opalescence; in time slight green precipitate.	Bluish opalescence; bluish-white fluorescence. In time, slight green precipitate.	Bluish-white opalescence, then slight green precipitate.
Concentrated copper chloride.	Turbidity, then greenish precipitate. Bluish-white fluorescence. Gradual change through grey, violet-grey, dark violet to (after 12 days) carmine.	Bright green precipitate. The liquid gradually becomes turbid.	Abundant greenish-white precipitate.	Abundant green precipitate.
Dilute copper sulphate.	No opalescence or fluorescence. Bluish-white precipitate.	Thick, flocculent, bluish-white precipitate.	Abundant bluish white precipitate; flocculent.	Bluish opalescence. Then slight blue precipitate.
Concentrated copper sulphate.	Immediate green coloration. Then abundant green precipitate.	Immediate precipitate abundant, bright blue.	Immediate greenish-white precipitate. Liquid slowly turns greenish-yellow.	Bright blue precipitate, fairly abundant.

	Methylaniline	Dimethylaniline	Ethylaniline	Diethylaniline
Dilute copper acetate.	Coloration: rose, dark rose, amber, dark brown. Then dark brown precipitate.	Bluish tint turning to lilac.	Brown coloration after a time.	Bluish opalescence. Then slight bluish precipitate.
Concentrated copper acetate.	Immediate emerald-green coloration, rapidly darkening to black. Then slight black precipitate.	Turbidity. Then green precipitate, which dissolves in strong alcohol to a bright blue liquid, becoming bright green, afterwards dark green.	Emerald-green coloration. Then bright green precipitate, soluble in strong alcohol. The solution after 4 hrs. turns dark green, and deposits the same precipitate.	Turbidity. Afterwards slight green precipitate.
Dilute cobalt chloride.	No coloration. In time precipitate of cobalt hydroxide.			
Concentrated cobalt chloride.	Violet-carmine coloration. After 4½ hrs., turbidity and deepening of the tint.			
Dilute nickel chloride.	Gradual turbidity but no precipitate even after a long time.			
Concentrated nickel chloride.	After 6 hrs. extremely slight green precipitate. Afterwards colour changes to dirty green, then to very dark brown.			

The presence of aniline and methylaniline is indicated by the rise of temperature produced on treating 5 c.c. of the dry oil with an equal measure of acetic anhydride. This is stated to be 0.815° for each unit per cent. of methylaniline present. For small percentages this appears to be fairly correct, but with a product actually containing 30%, an excess of over 7% is said to be indicated. A serious objection to the method is that it wholly fails in presence of aniline. But the presence of aniline can be recognised by mixing a few drops of the oil with a few drops of ether, and adding 1 drop of conc. sulphuric acid, when, if aniline be present, its sulphate will separate as a white precipitate.

A more plausible method is that of Nölting and Boasson (*Ber.*, 10, 795), based on the different behaviour of the bases with nitrous

acid,[1] but the results yielded in practice have been found unreliable by Reverdin and de la Harpe. These chemists recommend (*Chem. Zeit.*, 1889, **13**, 387, 407) for the estimation of the aniline and methyl-aniline conjointly, acetylation of the bases, and estimation of the excess of acetic anhydride by titration with alkali; and for the estimation of the aniline, diazotising and treating the product with beta-naphthol disulphonic acid.

Methylaniline and dimethylaniline may be distinguished in mixtures with each other according to H. Emde (*Arch. Pharm.*, 1909, **247**, 77–79) as follows.

The platinum salts can be used to distinguish between methylaniline and dimethylaniline in the presence of each other. Methylaniline platinic chloride, $(RHCl)_2PtCl_4$, forms short orange-coloured crystals, which decompose at 199° and can be crystallised from hot water containing hydrochloric acid. Dimethylaniline platinic chloride, $(RHCl)_2PtCl_4$, is produced by dissolving the base in strong hydrochloric acid and adding the solution to 10% platinum chloride solution. The orange-yellow precipitate is filtered off in the cold and recrystallised from alcohol containing hydrogen chloride. It then forms orange-red needles, decomposing at 173°. If a mixture of the bases is suspected, a small portion dissolved in acid is added to a platinum chloride solution, and the platinum double salts separated by crystallising from alcohol. If methylaniline only is to be looked for, the mixture can be crystallised from water. The presence of 5% of methylaniline in dimethylaniline can be recognised in this way.

At ordinary temperatures acetic anhydride has no action on dimethylaniline, but on prolonged heating tetramethyl-diamino-di-phenyl-methane is formed in considerable quantity, if the reagent be in excess. Methylaniline is converted into methyl-acetanilide, $C_6H_5.N(CH_3)(C_2H_3O)$, and aniline in the cold yields acetanilide, $C_6H_5.NHC_2H_3O$, but on heating more or less diacetanilide,

[1] When aniline hydrochloride is treated in cold solution with sodium nitrite, it yields diazobenzene chloride, whilst dimethylaniline is converted into the hydrochloride of its nitroso-derivative. Both these substances are freely soluble in water, while methyl-aniline is converted by the same treatment into the non-basic methylaniline-nitrosamine, which can be extracted by agitating the liquid with ether. If this reaction occurred in its simplicity, the monomethyl-aniline could be estimated from the weight of the nitros-amine left on evaporating the ethereal solution. But when this is distilled in a current of steam, in which the nitrosamine is volatile, a considerable quantity of nitrophenyl-methyl-nitrosamine, $C_6H_4(NO_2).N(NO)(CH_3)$, remains as a residue. This substance is clearly produced by the oxidation of the nitrosamine, and direct experiment shows that pure methyl-aniline, on treatment with excess of nitrous acid, is converted into it, to the exclusion of the simple nitrosamine. As the molecular weights of the two substances are materially different (181:136), the indefinite character of the reaction prevents the accurate estimation of the methylaniline (Reverdin and de la Harpe, *Chem. Zeit.*, 1889, **13**, 387, 407).

$C_6H_5.N(C_2H_3O)_2$, is produced. To avoid the formation of these secondary products the following method of working is recommended: From 1 to 2 grm. weight of the sample is mixed as rapidly as possible with an accurately known quantity (about twice its weight) of acetic anhydride, in a small flask fitted with a reflux condenser. After standing for half an hour at the ordinary temperature, 50 c.c. of water should be added, and the flask heated on the water-bath for 50 minutes to effect the conversion of the excess of acetic anhydride into acetic acid. The liquid is then cooled, diluted to a known volume, and an aliquot part titrated with standard alkali hydroxide, with phenolphthalein as an indicator.[1] By this means the excess of acetic anhydride, $C_4H_6O_3$, is ascertained, and the difference between the amount so found and that employed is the weight which has reacted with the *aniline* and *methylaniline* contained in the sample. 51 parts of acetic anhydride consumed in the reaction correspond to 107 of base in terms of *methylaniline*, and the percentage of base thus found (*a*) is calculated and recorded.

The *aniline* itself is estimated as follows: From 7 to 8 grm. of the sample are dissolved in hydrochloric acid (28 to 30 c.c.), and diluted with water to 100 c.c. 10 c.c. of this solution are further diluted with water and cooled by ice. The solution is then diazotised by adding a solution of sodium nitrite in quantity sufficient to react with the whole of the sample if it consisted of aniline solely. A solution of the sodium salt of β-naphthol-disulphonic acid known as "R Salt," is meanwhile prepared of a strength approximately corresponding to 10 grm. of naphthol per litre, and its precipitating power is calculated from its known strength, or exactly ascertained by experiment with pure aniline.

A measured quantity of this solution is now treated with excess of sodium carbonate, and to it the ice-cold solution of the diazotised sample is slowly added. Common salt is then added till a precipitate ceases to form, when the liquid is filtered, and portions of the filtrate are tested with R salts and the diazo-solution respectively, to ascertain which of these two is present in excess. Another experiment is then made with suitably varied volumes, until after a few trials exact precipitation of the colouring matter is attained without sensi-

[1] H. Giraud (*Bull. Soc. Chim.*, 1889, **2**, 142) modifies this process by employing the acetic anhydride dissolved in 10 times its volume of dimethylaniline. 10 c.c. of this solution are added to 1 grm. of the sample. After standing for 1 hour in a corked flask, water is added, and the liquid boiled for some time and titrated with standard barium hydroxide in the presence of phenolphthalein.

ble excess of either the naphthol or diazo-solution. The reactions which occur are as follows:

$$C_6H_5.NH_2,HCl + NHO_2 = C_6H_5N:N.Cl + 2H_2O; \text{and } C_6H_5.N_2Cl + C_{10}H_5(OH)(SO_3Na)_2 = HCl + C_6H_5.N_2.C_{10}H_4(OH)(SO_3Na)_2.$$

From these formulæ, and the volumes of the 2 solutions required for exact reaction, the weight of aniline present can be calculated. 1 grm. of R salt will react with 0.2672 grm. of *aniline.* The percentage of aniline thus found (*b*) is multiplied by 1.15 ($=^{107}/_{93}$), which gives its equivalent in methylaniline, and this (*c*) subtracted from the sum of aniline and methylaniline in terms of methylaniline found by the acetylisation process (*a*) gives the percentage of real *methylaniline* (*d*) present. The *dimethylaniline* is estimated by difference.

In the case of a sample of known composition, Reverdin and de la Harpe obtained the following satisfactory results by the foregoing process:

	Present	Found
Aniline,	10 42%	10.30%
Methylaniline,	10 97	11.16
Dimethylaniline (by difference)	78.61	78.54
	100.00	100.00

The presence of methylaniline is more objectionable in dimethylaniline intended for the manufacture of green than in that to be used for violet. Schoop (*Chem. Zeit.*, 1887, **11**, 254) states that the proportion seldom exceeds 2%, and that the best qualities of dimethylaniline are nearly or quite free from it. When present, monomethylaniline can be removed by shaking the oil with a small quantity of dilute sulphuric acid, or by boiling with acetic acid for 2 hours.

Diethylaniline. $C_6H_5.N(C_2H_5)_2$.

This base is prepared by heating 1 molecule of aniline hydrobromide with excess of ethyl alcohol to 145° for 8 or 10 hours in an autoclave. Nearly the theoretical yield is obtained. It is also made commercially by treating aniline with diethyl sulphate, the later product having now become available in quantity and at very low prices. The base boils at 216.5°. Diethyl-*o*-toluidine and diethyl-*p*-toluidine may be obtained by exactly similar means.

Diphenylamine. Phenylaniline. $C_6H_5.NH.C_6H_5$.

This base is obtained by heating aniline with the hydrochloride or other salt of aniline. 6 parts of aniline and 7 of aniline hydrochloride and a small amount of iodine are heated to 250° under a pressure of 4 or 5 atmospheres for 24 hours. The iodine seems to act as a catalyst, increasing the speed of reaction and also the yield. The ammonia formed is allowed to escape at intervals to prevent reconversion of the diphenylamine into aniline. The product is treated with warm hydrochloric acid and a large quantity of water, which dissolves any unchanged aniline hydrochloride, and decomposes the hydrochloride of diphenylamine, which latter base separates out and is purified by distillation. Diphenylamine crystallises in small white plates, having an agreeable flowery odour and burning taste. It melts at 54° and boils at 310°. It is almost insoluble in water, but readily soluble in alcohol, ether, benzene, and aniline. Diphenylamine has very feeble basic properties. The *hydrochloride* is a white crystalline powder, which turns blue in the air, and is decomposed by water. The most characteristic test of diphenylamine is the deep blue colour produced by adding a trace of nitric acid to its solution in strong sulphuric acid. The reaction, which is very delicate, is employed as a test for nitric acid.

The deep coloration produced by the action of nitric acid on diphenylamine sulphate, which has been looked upon as a characteristic reaction for nitric acid, is also, according to I. Bay (*Comptes Rend.*, 1905, **140**, 796–797), observed with other oxidising agents, and also by long exposure to the atmosphere. It is pointed out that, in general, all aromatic amines give rise to more or less highly coloured oxidation products.

Commercial diphenylamine should be pale yellow, melt not much below 54°, be free from unpleasant odour and oily matters, and give no violet coloration with bleaching powder. It is used for making Diphenylamine Blue, Aurantia, Orange IV and many other dyes.

Diphenylamine in alcoholic solution reacts with bromine to form tetrabromodiphenylamine (W. Dreger, *Z. ges Schiess- und Sprengstoff.*, 1909, **4**, 123) $(C_6H_5)_2NH + 8Br = (C_6H_3Br_2)_2NH + 4HBr$. This bromo-derivative is insoluble in water, sparingly soluble in alcohol, readily soluble in benzene, xylene, chloroform, and ethyl acetate, especially on warming; it melts at 102°, and crystallises in reddish needles having a silky lustre. For the estimation of

diphenylamine in the commercial product, the sample is dissolved in alcohol or, if already in ethereal solution, the ether may be driven off by adding alcohol and warming. Excess of bromine is then added, drop by drop, with constant stirring. The solution is then mixed with twice its volume of water, and the whole boiled until the alcohol and excess of bromine are driven off and the bulk is reduced to one-half, constant stirring being needed. The precipitate of tetrabromo-diphenylamine is transferred to a funnel, or Gooch crucible, connected with a water pump, and washed with warm water to remove the last traces of alcohol and bromine, and lastly dried at 98–100° until of constant weight. Smaller quantites may be estimated by evaporating to dryness in a previously weighed glass vessel. If it be desired to estimate the diphenylamine contained in gelatinised nitro-cotton, the nitro-compound is gradually decomposed by means of soda lye in a capacious flask. The flask is closed by a doubly perforated stopper fitted with a stoppered funnel, and a bent tube attached to a condenser, which communicates with a suitable receiver. The free diphenylamine and any camphor that may be present are carried into the receiver, which contains ether. The distillate is well shaken with common salt; the ether completely dissolves the diphenylamine and any camphor, and the estimation is then completed as before indicated.

Methyl-diphenylamine, $C_6H_5.N(CH_3)C_6H_5$, boils at 282°, and gives various colour-reactions with oxidising agents. In dilute sulphuric acid it dissolves to form a liquid of the colour of solution of potassium permanganate.

Warm nitric acid converts diphenylamine and its methyl-derivative into $C_6H_2(NO_2)_3.NH.C_6H_2(NO_2)_3$, hexanitro-diphenylamine, the ammonium salt of which constitutes the colouring matter known as *Aurantia* (Vol. 6).

p-Amino-diphenylamine results from the reduction of phenyl-amino-azobenzene, nitro-phenylamine, or Tropæolin OO (Vol. 6).

Triphenylamine. Diphenylaniline. $(C_6H_5)_3N$.

This substance is formed by the action of brombenzene on dipotassium aniline. It is a neutral substance, melting at 127°, and crystallising from ether in monoclinic pyramids. It forms no isonitrile, picrate, or acetyl compound, but yields iodide of triphenyl-methyl-ammonium on treatment with methyl iodide. Its solution in glacial acetic acid is coloured green on adding a little nitric acid, but with sulphuric acid it gives a violet coloration changing to blue.

Aminophenols

By the reduction of the nitrophenols, corresponding amino-compounds are obtained. These substances may also be prepared by heating either of the 3 isomeric amino-hydroxybenzoic acids, $C_6H_3(NH_2)OH.COOH$, with barium hydroxide.

In the aminophenols the acid character of the phenols is neutralised by the presence of the amino-groups, so that they only yield salts with acids; but as phenols they are still capable of yielding alkyl-derivatives (*e. g.*, anisidine), while the hydrogen of their amino-groups may be replaced for acetyl, etc., as in phenacetin.

The aminophenols form colourless crystalline scales or plates, which are very readily oxidisable on exposure to air, with blackening and formation of resinous products, especially if impure. On the other hand, their hydrochlorides are relatively stable, and, when pure, capable of sublimation. The solution of *p*-aminophenol hydrochloride is coloured first violet and then green by solution of bleaching powder, quinone chlorimine, $C_6H_4O(NCl)$, being formed; whilst with chromic acid mixture, and other oxidising agents, it yields quinone, $C_6H_4O_2$. Treatment with hydrogen sulphide and ferric chloride converts it into compounds of the *Methylene-Blue* group (Vol. 6).

The formyl and acetyl derivatives of the ortho-aminophenols are converted with great facility into anhydro-bases. Thus ethenyl-amino-phenol, a basic liquid boiling at 200 to 201°, is obtained by boiling ortho-aminophenol with acetic anhydride.

$$C_6H_4(OH).NHC_2H_3O = C_6H_4\left\langle\begin{matrix}N\\O\end{matrix}\right\rangle C.CH_3 + H_2O$$

When this substance is heated with dilute acids, the reverse action occurs, acetyl-*o*-aminophenol being formed.

The methyl esters of the aminophenols (anisidines or amino-anisoles) and the corresponding ethyl esters (phenetidine or aminophenetoles) are bases resembling aniline, and are employed for producing certain azo-dyes (*e. g.*, Anisole Red, Phenetole Red; Vol 6). The acetyl-derivatives of these esters are used in medicine under the names of *methacetin* and *phenacetin* (see below), the latter being widely used.

The following tables show the characters of the isomeric aminophenols and their derivatives:

	o-1:2		m-1:3		p-1:4	
	M. p.	B. p.	M. p.	B. p.	M. p.	B. p.
	°	°	°	°	°	°
Aminophenol (page 587) $C_6H_4\begin{cases}OH\\NH_2\end{cases}$	170	sub-limes	......	......	184	......
Acetyl-derivative $C_6H_4\begin{cases}OH\\NH(COCH_3)\end{cases}$	201	......	......	......	179	......
Methyl-ester (anisidine) $C_6H_4\begin{cases}O(CH_3)\\NH_2\end{cases}$	......	228	......	251	56	246
Ethyl-ester (phenetidine) $C_6H_4\begin{cases}O(C_2H_5)\\NH_2\end{cases}$	......	229	......	180–205 (at 100 mm.)	......	253
Methacetin (page 596) $C_6H_4\begin{cases}O(CH_3)\\NH(COCH_3)\end{cases}$	84	204	......	......	127	......
Phenacetin (page 590) $C_6H_4\begin{cases}O(C_2H_5)\\NH(COCH_3)\end{cases}$	70	....	97	......	135	......
Aminophenacetin. Phenocoll $C_6H_4\begin{cases}O(C_2H_5)\\NH(CO.CH_2.NH_2)\end{cases}$	......	......	......	......	100 5	......

An important member of this group is ortho-anisidine-1.amino-2.methoxy-benzene, 1-NH_2-2-OCH_3-C_6H_4, used as an intermediate in the manufacture of Cosamines, and Rhodines. It is made by reducing ortho-nitroanisole with iron dust and a little acid (*Method of analysis*).

Commercial Method[1] for the Analysis of *o-Anisidine.*

$C_6H_4.OCH_3.NH_2$ 1.2, M. W. 123.11.

Solubility.—Pipette a 5 c.c. sample into a 400 c.c. beaker, add 10 c.c. conc. hydrochloric acid and 200 c.c. water. Stir to dissolve, and when cool examine for turbidity and oil on the surface.

Moisture.—Weigh 200 grm. of the sample into a 500 c.c. distilling flask (with outlet tube 1 "below top of neck"). Add 100 c.c. water-saturated toluol and distil the water and toluol into a 120 c.c.

[1] The Newport Company—Carrollville Laboratory.

separatory funnel, with stem graduated in 0.1 c.c. divisions. Measure the water as the lower layer.

$$\frac{\text{c.c. water} \times 100}{200} = \%\ \text{Moisture}$$

o-Anisidine.—Weigh approximately 5 grm. of the sample accurately in a weighing bottle. Transfer into a 500 c.c. volumetric flask, washing the bottle with water and 10 c.c. conc. hydrochloric acid, and dilute to volume.

Pipette 50 c.c. of this dilution into a 500 c.c. beaker, dilute to 300 c.c., and add 30 c.c. conc. hydrochloric acid. Titrate at 30° with N/10 sodium nitrite until, after standing 10 minutes, a faint reaction for excess nitrite is obtained with starch iodide when spotted on a white porcelain spot plate.

$$\frac{500 \times \text{titration} \times \text{N/10 nitrate factor} \times 0.012311 \times 100}{\text{Wt. of sample} \times 50} = \text{Ortho-anisidine}$$

Standard Nitrite Solution.—Dissolve 7.25 grm of commercial, approximately 95% sodium nitrite per litre of water and standardise by titrating in the same manner at 15°–20° C. against recrystallised sulphanilic acid.

Estimate the purity of the sulphanilic acid by a Kjeldahl nitrogen determination.

Distillation.—Measure in a graduated cylinder 100 c.c. sample, pour into a dry 200 c.c. M. C. A. Pyrex distilling flask, and do not rinse out the cylinder. Support the flask on a 6″ × 6″ asbestos board, 1/8″ thick, with a hole in the centre 1 1/4″ in diameter. Support the board on a circular shield enclosing the flame. Insert through a cork stopper, a 14″ thermometer, with the top of the auxiliary bulb opposite the middle of the vapour tube. The thermometer shall be graduated in 1/5° have a range 200°–250° and be standardised totally immersed. Heat the flask with the short flame of a Bunsen burner. Distil at the rate of 1 drop per second through a straight, air-cooled condenser tube, 24″ long and 1/2″ internal diameter, so placed that the vapour tube will extend 1″ into the narrow portion. Receive the distillate in the same cylinder used for measuring the sample.

Note the temperatures at 5 c.c. and 90 c.c. and apply the following corrections:

(*a*) Correction on thermometer, determined by standardisation.

(*b*) Stem correction, determined from the equation

$C = E(T\text{-}t)\ 0.00016$, in which

E = Degrees from centre of stopper to point of reading.

T = Temperature reading.

t = Room temperature surrounding thermometer at point of reading.

C = Stem correction, additive.

(*c*) *Barometer Correction.*—For each mm. difference between 760 and the observed corrected barometric pressure, add or subtract 0.062°, depending upon whether the observed pressure is below or above 760 mm.

Phenacetins. Acet-phenetidins.

$$C_6H_4 \begin{cases} O(C_2H_5) \\ NH(CO.CH_3) \end{cases}$$

The substances of this formula have acquired value as antipyretics and analgaesics.

The phenacetins are prepared by ethylating the corresponding mononitrophenols, thus obtaining the isomers of the formula $C_2H_2(NO_2).OC_2H_2$. On treatment with zinc or iron and hydrochloric acid, these are reduced to the corresponding phenetidines, $C_2H_2(NH_2).OC_2H_2$, which are purified and acetylised by heating with glacial acetic acid for some hours, the products being recrystallised from water.

Of the 3 isomeric phenacetins, the *m*-compound is unimportant. It forms tasteless and odourless scales, melting at 96°.

Para-acetphenetidin is the official variety in the *German, British,* and *United States Pharmacopœias*. It forms white, odourless, tasteless, glistening scaly crystals. It requires 1400 parts of cold, or 70 parts of boiling, water for solution, and is soluble to a notable extent in chloroform. Its solution in 16 parts of alcohol is precipitated by the smallest addition of water. The crystals melt at 135°.

Ortho-acetphenetidin forms brilliant white, very light spangles, without taste or odour, and melting at 70°. It is very slightly soluble in cold, but more readily in hot, water, separating again on cool ing. It dissolves in about 3 parts of rectified spirit, and abundantly in chloroform.

Besides the differences in their m. p. and solubilities, *p*- and *o*-phenacetin are distinguished by their behaviour when boiled for

several hours with dilute sulphuric acid (sp. gr. 1.26). When thus treated, the para-compound yields acetic acid and sparingly soluble sulphate of phenetidin. Orthophenacetin, on the other hand, is not decomposed by the same treatment, requiring the action of acid of 1.575 sp. gr. for 2 hours at 90° to effect its hydrolysis.[1] If in either case the acid liquid be diazotised, and then treated with an ammoniacal solution of naphthol-disulphonic acid, a fine red-yellow colour will be obtained if *p*-phenacetin was employed, whilst with the *o*-compound a cherry-red coloration is produced. In either case the colouring matter may be precipitated by brine.

This formation of an azo-colouring matter may be employed to detect the phenacetins in urine and other organic liquids. The urine is evaporated to dryness, and the residue treated with hot alcohol. The solution is filtered, evaporated, and the residue boiled for 2 hours with dilute sulphuric acid (sp. gr. 1.26) under a reflux condenser. The resulting solution is cooled to 5 or 6°, treated with a 1% solution of sodium nitrite for 5 minutes, and then poured into a solution of naphthol-disulphonic acid in excess of ammonia, care being taken that the mixture remains alkaline. If either modification of phenacetin be present in the urine, a characteristic coloration will be produced, from the intensity of which the amount of phenacetin may be estimated.

For medicinal use, phenacetin is said to present considerable advantages over antipyrine, and especially over antifebrin (acetanilide), for while the latter compound is decomposed in the system with formation of aniline, which has marked toxic properties, phenacetin yields phenetidin, $C_6H_4(OC_2H_5).NH_2$, and aminophenol, $C_6H_4(OH).NH_2$, which are said to be harmless. Paraphenacetin, in doses ranging from 8 to 20 grains for adults, and from 2 to 3 grains for children, is said to be valuable antipyretic and antineuralgic, without producing nausea, vomiting, cyanosis, or disagreeable after-effects. Being nearly insoluble, it is best given in the form of powders. The dose of *o*-phenacetin required to produce the same effect is larger than that of the *p*-compound.

According to Reuter (*Pharm. Zeit.*, 1891), phenacetin is liable to contain unconverted *p-phenetidin*, which appears to be poisonous in very small doses, if taken for some time, producing nephritis and

[1] S. Lüttke detects *o*-phenacetin by boiling 15 grm. of the sample with 25 grm. of dilute hydrochloric acid, when *o*-phenetidin hydrochloride is formed, from which the free base may be separated by sodium hydroxide, and its b. p. (given by Luttke as 242.5°) determined. The hydrochloride gives a blood-red coloration with ferric chloride.

albuminuria. To detect the impurity, Reuter melts 2.5 grm. of chloral hydrate at 100°, and adds 0.5 grm. of the sample. On agitation the phenacetin dissolves, and, if pure, the solution will remain colourless when heated on the water-bath for 5 minutes, though after longer heating it will assume a rose tint. In presence of para-phenetidin an intense coloration, ranging from red-violet to blue-violet, is produced in 2 or 3 minutes at most.

S. Lüttke detects *diaminophenols* or *diaminophenetoles* in phenetidine by grinding 0.5 grm. of bleaching powder to a fine paste with hydrochloric acid, and adding about 0.03 of the sample, when a red colour will be produced.

G. M. Beringer (*Amer. Pharm. Assoc.*, 1903; *Chem. and Druggist*, 1903, **63**, 377) suggests the following test. 0.1 grm. of the phenacetin is boiled with 3 c.c. of a 50% solution of sodium hydroxide for 1 minute, then cooled and shaken with 5 c.c. of sodium hypochlorite solution. If the sample be pure, a clear yellow liquid is obtained, but if acetanilide be present, a purple-red or brownish-red turbidity or precipitate is produced.

The lower price of *acetanilide*, and its close physical resemblance to phenacetin, have suggested the possibility of the partial or complete substitution of the former compound for the latter, and a flagrant instance of such a practice is actually on record (*Pharm. Jour.*, [iii], **21**, 377). The presence of 5% of acetanilide lowers the m. p. of the sample to 127–128°.

H. Schwartz (*Pharm. Journ.*, [iii], **18**, 1085) recommends that 1 grm. of the suspected sample should be heated with 2 c.c. of sodium hydroxide solution, a fragment of chloral hydrate or a few drops of chloroform added, and the mixture again gently heated. With phenacetin the odour is aromatic and not disagreeable, but in presence of acetanilide, the penetrating and repulsive smell of phenylcarbamine, $C_6H_5.NC$, is produced. On boiling the sample with sodium hydroxide solution, oily drops of aniline separate if acetanilide be present in considerable quantity. If the cooled liquid, together with the separated globules, be shaken with ether, and the ether separated and evaporated, the residue when dissolved in water and treated with a drop of carbolic acid, and a clear solution of bleaching powder added, gives a blue-green coloration changed to onion-red by hydrochloric acid, and restored by ammonia (See also *J. Soc. Chem. Ind.*, 1888, **7**, 772).

The following tests for distinguishing acetanilide from phenacetin have been suggested by E. Barral (*J. Pharm. Chim.*, 1904, **19**, 237).

Phosphomolybdic acid reagent gives a bright yellow precipitate in an aqueous solution of acetanilide, which is soluble on heating. Phenacetin gives a similar precipitate which is insoluble on heating.

Mandelin's reagent (1 grm. of ammonium vandate in 200 grm. of sulphuric acid) gives a red colour, changing rapidly to greenish-brown with acetanilide. With phenacetin the colour is olive-green while cold and reddish-brown after being warmed.

Barral has also suggested the following additional colour tests for phenacetin. *Sodium persulphate* produces a yellow colour when warmed with phenacetin; when boiled for some time the colour becomes orange.

Bromine water, when heated in contact with a few crystals of phenacetin, colours the latter rose; the liquid becomes orange-yellow, and a brown precipitate is gradually formed on cooling.

Millon's reagent, when heated in contact with phenacetin, turns yellow and then red; nitrous fumes are finally formed.

For the detection of acetanilide in phenacetin, M. J. Schröder recommends that 0.5 grm. of the sample should be boiled with 8 c.c. of water, and the liquid filtered when cold from the recrystallised phenacetin. The filtrate is boiled with a little potassium nitrite and dilute nitric acid, a solution of mercurous nitrate containing a little nitrous acid added, and the whole again boiled. A red colour will be obtained if the proportion of acetanilide in the sample exceeds 2%.

If 1 grm. of a mixture of equal parts of phenacetin with acetanilide be shaken with 200 c.c. of water, the whole of the acetanilide goes into solution together with 0.130 grm. of phenacetin, whilst the remainder of the phenacetin remains insoluble. If this be separated, its weight, when corrected by an addition of 0.130, will represent the phenacetin present in 1 grm. of the sample (*Pharm. Jour.*, [III], **21**, 377).

Phenacetin is distinguished from *exalgin* and *antifebrin* by boiling 0.1 grm. for a minute with 1 c.c. of hydrochloric acid, adding 10 c.c. of water, filtering, and adding to the filtrate 3 drops of a 3% solution of chromic acid, when a ruby-red colour will be gradually developed (See *Pharm. J.*, [III], **21**, 978). Strong sulphuric acid should dissolve phenacetin without becoming coloured, whilst a saturated solu-

tion, if free from phenol and acetanilide, will not become turbid on adding bromine-water.

TABLE OF TESTS FOR ACETANILIDE AND PHENACETIN

Comparative	Acetanilide	Phenacetin
M. p	112°	135°
Boils.	295°	. . . .
Solubilities at 25°	2.5 pts. alcohol. 0.4 pts. boiling alcohol. 5 pts. chloroform. 179 pts. water. 18 pts. boiling water. Solution conc. H_2SO_4 without change of colour.	12 pts. alcohol. 2 pts. boiling alcohol. 925 pts. water. 70 pts. boiling water. 63 pts. ether. 20 pts. chloroform. Solution conc. H_2SO_4 without change of colour.
Phenol and bleaching powder solution. (See page 539.)	Behaves like aniline.	No reaction.
Iso-nitrile test with alkali hydroxide and chloroform. (See page 538.)	Odour of *iso*-nitrile at once.	Odour of *iso*-nitrile only on standing a few minutes.
Boiling with a strong solution of sodium hydroxide.	Oily drops (aniline separates).	No separation.
Addition of sodium nitrite and dilute nitric acid boiled.	Red colour.	No colour.
Then mercurous nitrate added and boiled.	Bright yellow ppt. soluble on heating.	Bright yellow ppt. insoluble on heating.
Phosphomolybdic acid to aqueous solution.	. .	. . .
Mandelin's reagent in aqueous solution.	Red colour changing rapidly to greenish-brown.	Olive-green colour while cold and reddish-brown after being warmed.

No single reliable method has been so far known for the estimation of acetanilide and phenacetin in complex mixtures. The following method of J. L. Turner and C. E. Vanderkleed (*Amer. J. Pharm.*, 1907, **79**, p. 521), however, is said to give good results. It is based on the saponification of acetanilide by an alkali, distillation of acetic acid from the resulting acetate by means of a phosphoric acid solution, and titration of the distillate. It is carried out as follows: 1 grm. of acetanilide is saponified by heating to boiling under a reflux condenser, for 1½ to 2 hours, with 3 grm. of sodium hydroxide, .020 grm. of alcohol and .010 grm. of water. The solution is transferred to an evaporating dish, and the alcohol driven off on the water-bath. The residue is transferred to a separator and shaken out once with ether in order to remove the aniline separated from the acetanilide. The ethereal solution is shaken out twice with water

to remove traces of sodium acetate, which is somewhat soluble in ether, and the washings added to the original aqueous residue. The aqueous solution is then transferred to a flask of 1 litre capacity, acidified with 0.025 grm. of 85% phosphoric acid, and the acetic acid completely distilled off with steam, this being shown when no further reaction is given with litmus. The distillate is then titrated with N/1 sodium hydroxide, with the addition of 0.001 grm. of 1% solution of phenolphthalein. 0.001 grm. of N/1 sodium hydroxide = 0.13409 grm. of acetanilide. Phenacetin, being closely allied to acetanilide, is estimated in exactly the same way, 1 mgrm. of N/1 sodium hydroxide being equivalent to 0.17779 grm. of phenacetin. The two substances, however, cannot be estimated when present in the same preparation. Acetates, nitrates, and nitrites interfere with the method. These salts are removed by means of chloroform extraction; but when they are present in the phosphoric acid, or in the alkali used for saponification, they must first be removed. Chlorides do not interfere, but if carbonate be present in the alkali used for saponification, the carbon dioxide formed on the addition of phosphoric acid would be distilled over with the acetic acid and vitiate the result of the titration; it is therefore advisable to heat the acidified solution to boiling under a reflux condenser before distillation, in order to drive off the carbon dioxide.

Methyl-phenacetin, $C_6H_4(O.C_2H_5).N(CH_3)(C_2H_3O)$.—This substance is prepared by treating *p*-phenacetin in xylene solution with sodium, and causing the resultant sodium-derivative to react with methyl iodide (*Pharm. Jour.*, [III], **21**, 81). The new product distils at about 300° as an oil, which crystallises on standing. It may be purified by recrystallisation from alcohol or ether, when it forms colourless crystals, moderately soluble in water, and having marked narcotic as well as antipyretic characters.

Amino-p-phenacetin, $C_6H_4(O.C_2H_5).NH(CO.CH_2.NH_2)$.—The *hydrochloride* of this base is readily soluble in water and alcohol, and has been introduced, under the name of "*phenocollum hydrochloricum,*" as an antipyretic and antirheumatic. Prolonged boiling with alkalies splits it into *p*-phenetidine and glycine.

Para-phenetol carbamine, Dulcin, $CO(NH_2)NH.C_6H_4OC_2H_5$. Colourless crystals, m. p. 173°. Sparingly soluble in cold water, much more so in hot water, alcohol and ether. This substance is 200 times as sweet as sugar.

Formyl-p-phenetidine $C_6H_4(O.C_2H_5).NH(CO.H)$, though having a constitution similar to acetphenetidin, appears to have no anti-pyretic properties, but has been suggested as an antidote in cases of poisoning by strychnine.

Para-diethoxyethenyldiphenylamide $CH_3C:(N.C_6H_4OC_2H_5)NH.-C_6H_4OC_2H_5$. It is a white crystalline powder, insoluble in water, m. p. 121°. The hydrochloride, m. p. 189° is more used as a local anæsthetic.

Methacetin is the commercial name of *p*-acetanisidine, $C_6H_4(O.CH_3).NH.C_2H_3O$. It is, consequently, the lower homologue of phenacetin. It forms a crystalline powder or small lustrous scales or plates, odourless, but of a faintly bitter taste. It melts at 127°, and at a higher temperature boils and distils unchanged. It dissolves in 526 parts of cold, or 12 of boiling, water, and is easily soluble in alcohol, acetone, chloroform, and dilute acid and alkaline liquids. It is less soluble in benzene, and only with difficulty in ether, carbon disulphide, petroleum spirit, and oil of turpentine, but dissolves freely, on warming, in glycerin and fixed oils. In its general reactions and physiological effects, metacetin closely resembles phenacetin, though according to some authorities it has a less powerful, and according to others a more powerful, action. Its efficacy in cases of neuralgia and rheumatism is said to greatly exceed that of phenacetin, from which it may be distinguished by its physical characters, or by heating it with a quantity of water insufficient for its solution. When thus treated, methacetin melts and solidfies again on cooling, whereas phenacetin undergoes no apparent change. 1 c.c. of hydrochloric acid dissolves 0.1 grm of methacetin very easily, whereas the same quantity of phenacetin is mainly undissolved.

Diaminophenols. $C_6H_3(OH)(NH_2)_2$.

These substances are weak bases, forming salts which crystallise well and give aqueous solutions which turn brown in the air; and are coloured an intense violet or dark red by potassium dichromate, ferric chloride, or bleaching powder. The 1–2–4 diaminophenol is sold in the form of its sulphate and is a widely used photographic developer.

Triaminophenol. $C_6H_2(OH)(NH_2)_3$.

This substance is an unstable base resulting, from the complete reduction of picric acid, $C_6H_2(OH)(NO_2)_3$, in acid solutions. If alkaline reducing agents be employed, the action does not proceed beyond the formation of dinitro-amino-phenol or picramic acid,

$C_6H_2(OH)(NH_2)(NO_2)_2$. A dilute solution of triaminophenol is coloured deep blue by ferric chloride.

Phenylene-diamines. Diaminobenzenes

Three modifications of phenylene-diamine or diaminobenzene, $C_6H_4(NH_2)_2$, are known, differing from each other in properties according to the positions of the amino-groups, thus:

	Ortho-compound 1:2	Meta-compound 1:3	Para-compound 1:4
Appearance	Tablets or plates ..	Crystalline mass.....	Tablets or small plates
M. p	102–103°	63°	140°
B. p	252°	287°	267°
Characters of hydrochloride.	Groups of radiating needles; readily soluble.	Concentrically arranged crystals.	Readily soluble tablets; very sparingly soluble in hydrochloric acid.
Reaction in neutral solution with sodium nitrite.	Separation of amino-azo-phenylene as a colourless oily liquid.	Yellow or brown coloration, or precipitate of triamino-azo-benzene.	No reaction.

Commercial Method[1] for the Analysis of *Meta-phenylene-diamine.* $C_6H_4(NH_2)_2$ 1.3 M.W. 108.11.

1. *Solubility.*—Pour about 5 grm. of the molten sample into 500 c.c. water, stir to dissolve and examine for solubility.

2. *Base* (*Preparation of* 0.04 *N diazobenzene*).—Prepare a stock solution of aniline hydrochloride by dissolving 9.3094 grm. of pure aniline[2] in 45 c.c. conc. HCl acid and sufficient water to make up to one liter.

Pipette 100 c.c. of this stock solution into a 250 c.c. volumetric flask. Cool the flask by surrounding it with an ice and salt mixture until the contents are frozen, then add, while shaking, 105 to 110 c.c. 0.1 N sodium nitrite solution. Allow the solution to stand 30 to 40 minutes, dilute to the mark with ice water and shake. Keep the solution at 0°, protected from light, and do not use after 2 hours old.

[1] From the Newport Company—Carrollville Laboratory.

[2] Distil the best grade of commercial aniline *in vacuo*, collect the constant-boiling middle fraction and protect the distillate from moisture. Redistil until a product is obtained with a freezing point of minus 6.30° or above.

3. *Procedure.*—Pipette approximately 4 grm. of the melted sample into a tared weighing bottle and weigh. Immediately add about 10 c.c. water to the weighing bottle, then transfer to a beaker and add about 300 c.c. water and 10 c.c. conc. HCl. Transfer to a 500 c.c. volumetric flask and dilute to volume.

Pipette 25 c.c. of this solution into a 600 c.c. beaker, add water and clean ice to make a volume of 200 c.c. and 5–10 grm. sodium acetate. Place the solution under a mechanical agitator and keep at 0–5° with an ice bath. Add about 75% of the required amount of 0.04 N diazo-benzene from a jacketted burette cooled with ice. Follow the titration by spotting out on a pinch of salt on good drop test paper. Test on one side with diazo solution and on the other side with a portion of the phenylene-diamine solution from the flask, to which has been added a few grm. of sodium acetate, and observe the orange rings. When the ring ceases to appear on the diazo side add 10–15 grm. salt and continue the titration until a faint orange ring is obtained with phenylene-diamine. If the orange ring again appears (when tested as above) after the solution has stood 5 minutes, the titration is finished.

$$\frac{\text{c.c. diazo solution} \times 0.0043244 \times 100 \times 500}{\text{Wt. of sample} \times 25} = \%$$

4. *Crystallising Point.*—Nearly fill a 1″ × 4″ Pyrex test tube with melted sample, insert a standardised M. C. A. R-2, thermometer, range −10 to +80°. Place the tube in a 4 oz. bottle with mouth 114″ in diameter. Stir rapidly with the thermometer while holding the 76 mm. mark at the surface and avoid rubbing the walls as much as possible. After slight super-cooling, crystallisation will proceed, accompanied by a rise in temperature. If the sample should supercool to 50° before crystallisation starts, introduce a seed crystal. Note the highest temperature obtained, and apply any thermometer correction found by standardisation when immersed to the 76 mm. point. This temperature is taken as the crystallising point.

o-Phenylene-diamine is distinguished from its isomerides by its reaction with sodium nitrite, and by the separation of ruby-red needles on adding ferric chloride to the solution of its hydrochloride. On treating an alcoholic solution of the base with a drop of phenanthraquinone dissolved in glacial acetic acid, and boiling for a short time, a bright yellow precipitate of diphenylene-quinoxaline, $C_{20}H_{12}N_2$,

is formed. It consists of small needles which are coloured a deep red by strong hydrochloric acid, and its production affords the most delicate reaction for ortho-phenylenediamine. Its isomerides do not give the reaction, but its homologue, *o*-toluylenediamine, behaves similarly.

Meta-phenylene-diamine may be prepared by the reduction of meta-dinitrobenzene (Vol. IV). It often remains in a state of superfusion for some time, but is instantly solidified by adding a crystal of the solid substance. *m*-Phenylene-diamine is very soluble in water, the solution being alkaline in reaction. It is readily soluble in ether, and may be extracted by this solvent from alkaline aqueous liquids. It is a di-acid base, the *hydrochloride* being $C_6H_4(NH_2)_2,2HCl$. The reaction of *m*-phenylene-diamine with sodium nitrite is characteristic and extremely delicate. It is due to the formation of Bismarck or Phenylene Brown (Vol. 6), and by means of it 1 part per million of nitrous acid can be detected in water.

m-Phenylenediamine possesses marked poisonous properties, its physiological action resembling that of the leucomaines and ptomaines. Dubois and Vignon (*Compt. Rend.*, **107**, 533) experimented on dogs, and found that a dose of 0.1 grm. per kilogrm. of the animal produced salivation, vomiting, diarrhœa, abundant excretion of urine at intervals, and death by coma in 12 to 15 hours. Besides these severer symptoms, all those of intense influenza were produced, such as acute coryza and sneezing, coughing, and extreme depression.

p-Phenylene-diamine occurs in aniline tailings. It may be prepared by the reduction of *p*-nitracetanilide. It is but slightly soluble in cold water, but readily soluble in alcohol and ether. When heated with dilute sulphuric acid and manganese dioxide it yields quinone, $C_6H_4O_2$, which reaction distinguishes it from its isomerides. On passing hydrogen sulphide through a solution of the hydrochloride, and then adding ferric chloride, thionine or Lauth's Violet is formed (Vol. 6).

Para-phenylene-diamine possesses poisonous properties similar to those of meta-phenylene-diamine, but death occurs more rapidly than with the latter base. It also exerts a special action on the eye, which is gradually forced out of its orbit by the swelling of the conjunctiva or intra-orbital cellular tissue; while the lachrymal glands are blackened by a deposit of pigment (compare "Toluylene-diamines").

COMMERCIAL METHOD FOR THE ANALYSIS[2] OF META-TOLUYLENE-DIAMINE. $C_6H_3.CH_3.(NH_2)_2$ 1.2.4 M. W. 122.13

1. *Moisture.*—Weigh a 2.0 grm. sample into an ignited, weighed low form 50 c.c. porcelain crucible. Dry to constant weight over conc. H_2SO_4 *in vacuo.* Loss in weight is moisture.

$$\frac{\text{Loss in wt.} \times 100}{2} = \%\ \text{moisture}$$

2. *Ash.*—Take the crucible containing the dry sample from 1, ignite until free from carbonaceous matter, cool and weigh.

$$\frac{\text{Residue} \times 100}{2} = \%\ \text{ash}$$

3. *Base.—Preparation of* 0.04 *N diazo-benzene.*

Prepare a stock solution of aniline hydrochloride by dissolving 9.3094 grm. of pure aniline[1] in 45 c.c. conc. HCl acid and sufficient water to make up to one litre.

Pipette 100 c.c. of this stock solution into a 250 c.c. volumetric flask. Cool the flask by surrounding it with an ice and salt mixture until the contents are frozen.

Procedure.—Weigh 4.8852 grm. sample on a watch glass, wash into a beaker, dissolve in about 300 c.c. water containing 10 c.c. conc. HCl acid, and transfer to a 500 c.c. volumetric flask.

Pipette 25 c.c. of this solution into a 600 c.c. beaker, add water and clean ice to make a volume of about 200 c.c. and 5–10 grm. sodium acetate. Place the solution under a mechanical agitator and keep at 0°–5° with an ice bath. Add about 75% of the required amount of 0.04 N diazo-benzene from a jacketted burette cooled with ice. Follow the titration by spotting out on a pinch of salt on good drop test paper. Test on one side with diazo solution and on the other side with a portion of the toluylene-diamine solution from the flask to which has been added a few grm. of sodium acetate

[1] Distil the best grade of commercial aniline *in vacuo*, collect the constant-boiling middle fraction, and protect the distillate from moisture. Redistil until a product is obtained with a freezing point of minus 6.30° or above. Then add, while shaking 105 to 110 c.c. 0.1 sodium nitrite solution. Allow the solution to stand 30 to 40 minutes, dilute to the mark with ice water and shake. Keep the solution at 0°, protected from light and do not use after 2 hours old.

[2] The Newport Company—Carrollville Laboratory.

and observe the orange rings. When the ring ceases to appear on the diazo side, add 10–15 grm. salt and continue the titration until a faint orange ring is obtained with toluylene-diamine. If the orange ring again appears (when tested as above) after the solution has stood 5 minutes, the titration is finished.

$$\text{c.c. diazo used} \times 2 = \%$$

4. *Crystallising Point.*—Place portions of the sample in a 1″ × 4″ Pyrex test tube and heat over the direct flame until just melted and until the tube is nearly full of melted sample. Insert a standardised M. C. A. R-3 thermometer, range $-70°$ to $160°$. Place the tube in a 4 oz. bottle with mouth 1¼″ in diameter. Stir rapidly with the thermometer while holding the 76 mm. mark at the surface, and avoid rubbing the walls as much as possible. After slight supercooling, crystallisation will proceed accompanied by a rise in temperature. Note the highest temperature obtained and apply any thermometer correction found by standardisation when immersed to the 76 mm. mark. This temperature is taken as the crystallising point.

Dimethyl-p-phenylene-diamine, $H_2N.C_6H_4.N(CH_3)_2$, may be obtained by the reduction of nitrosodimethyl-aniline or of Helanthin (Vol. VI). A neutral solution of the hydrochloride is coloured a beautiful purple by ferric chloride; and on treating it with a hydrochloric acid solution of hydrogen sulphide, and then adding ferric chloride till the smell of sulphuretted hydrogen has disappeared, a fine blue coloration is obtained, due to the formation of Methylene Blue (Vol. 6).

Toluylene-diamines. Diaminotoluenes. $C_6H_3(CH_3)(NH_2)_2$. These bases closely resemble the phenylene-diamines. The *orotho-para*-modification ($CH_3:NH_2:NH_2 = 1:2:4$) is obtained by the reduction of ordinary dinitrotoluene. It melts at 99°, is used for the production of Toluylene Red and Toluylene Orange. The 1:3:4 (*meta-para*) modification is obtained by nitrating acet-*p*-toluide, saponifying, and reducing.[1] Janovsky (*J. Soc. Chem. Ind.*, **9**, 383) gives the following table of reactions of neutral or slightly acid solutions of the two isomeric toluylene-diamines:

[1] This modification appears to be identical with the *p*-toluylene-diamine isolated by Hell and Schoop from aniline tailings (*Ber.*, 1881, **12**, 723).

Reagent	α-Toluylene-diamine $CH_3 : NH_2 : NH_2 = 1:2:4$	β-Toluylene-diamine $CH_3 : NH_2 : NH_2 = 1:3:4$
Ferric chloride........	No change at first; after standing for a long time an orange coloration.	Wine-red coloration.
Potassium dichromate.	Yellowish-brown coloration.	Reddish-brown precipitate.
Potassium ferricyanide.	Olive-green crystalline plates.	Dark-red coloration.
Bromine water	Yellowish-white precipitate.	Brown flocks and magenta-red solution.
Platinic chloride.......	Yellowish-brown colouration.	Reddish-brown precipitate.
Auric chloride....... ..	Brown precipitate.	Red solution with blue reflex and metallic mirror in the cold.
Potassium nitrite.. ..	In very dilute solutions a golden brown coloration; in concentrated a brown precipitate.	No coloration, but a salmon-coloured precipitate.
Solution of bleaching powder.	Reddish-brown coloration and then a light brownish-yellow precipitate.	Dark-red coloration, then an olive-green precipitate.

The foregoing reactions are available, even in presence of other substances, for the detection and identification of the toluylene-diamines, which often result from the reduction of azo-dyes.

The toluylene-diamines are powerful poisons (compare "*m*-phenylenediamine," page 106).[1]

Benzidine.[2] Di-*p*-amino-diphenyl.

$$C_{12}H_{12}N_2 = NH_2.C_6H_4.C_6H_4.NH_2(1, 4:1, 4).$$

This substance is obtained by the reduction of diparanitrodiphenyl, $NO_2.C_6H_5.C_6H_4NO_2$, by nascent hydrogen (tin and hydrochloric acid). A readier method of preparation is the following: An alcoholic solution of 10 parts of azobenzene, $C_6H_5,N:N.C_6H_5$, is treated with a solution of 3.5 parts of tin in concentrated hydrochloric acid, and the liquid warmed for some time. Hydrazobenzene, $C_6H_5.NH.NH.C_6H_5$, is formed, which by intramolecular change is converted into benzidine (dihydrochloride). Some of the isomeric *o-p*-diamino-diphenyl is simultaneously formed, and a portion of the azobenzene is reduced to aniline, $C_6H_5.NH_2$. The

[1] Engel and Kiener (*Compt. Rend.*, 1887, **105**, 465) find the symptoms to vary considerably according to the time required to produce death, which ranges from a few hours in acute cases to several weeks in chronic cases. When death ensues in a few days, there is always icteria, and often hæmoglobinuria, and the urine is loaded with fat and yellow and brown pigment-granules, which sometimes contain iron. This ferruginous pigment accumulates in the spleen and marrow, and seems to be formed from the hæmoglobin in the protoplasm from the cellules, and not from the red corpuscles.

[2] The Newport Company—Carrollville Laboratory.

alcohol is distilled off, the residue dissolved in water, and sulphuric acid added. The nearly insoluble benzidine sulphate is precipitated, while the sulphates of the isomeric base and of aniline remain in solution. The precipitate is washed with dilute hydrochloric acid (to remove tin salts) and treated with ammonia, the liberated benzidine being crystallised from dilute alcohol. Benzidine is also produced by treating azobenzene with sulphur dioxide. Benzidine is manufactured on a large scale by heating nitrobenzene with sodium hydroxide, a little alcohol, and the proportion of zinc-dust theoretically sufficient to reduce it to hydrazo-benzene. The product is washed with cold dilute hydrochloric acid to remove oxide of zinc. On subsequently heating it with dilute hydrochloric acid, it is converted into benzidine dihydrochloride.

Benzidine forms large pearly plates, which are colourless when pure, but rapidly turn red on exposure to the air. It melts at 127.5 to 128° C. and boils with partial decomposition at 400 to 401° C. under 740 mm. pressure. Benzidine is very sparingly soluble in cold, but readily in boiling water, and is easily soluble in alcohol and ether.

Benzidine is a well-defined di-acid base, forming crystallisable salts. The sulphate is very sparingly soluble in water, even when boiling.

On adding potassium dichromate to a concentrated solution of benzidine hydrochloride, a deep blue crystalline precipitate, containing $C_{12}H_8(NH_2)_2CrO_4$, is immediately formed. The same precipitate is formed on warming, even in very dilute solutions.

When chlorine-water is added in small quantity to a solution of benzidine hydrochloride, the liquid assumes a fine blue colour, which on further addition of chlorine, water changes, to green; and ultimately, when the chlorine is in excess, a flocculent red precipitate is formed, apparently containing $C_{12}H_7Cl_3N_2O$, soluble in alcohol and ether, and forming a colourless compound on reduction. Bromine water and a solution of bleaching powder act similarly; but in presence of a large quantity of free hydrochloric acid bromine forms tetrabrombenzidine, melting at 285°. With nitrous acid, solutions of benzidine salts react to form tetrazo-compounds which react with phenols, phenol-sulphonic and carboxylic acids, aminosulphonic acids, etc., to form the important class of bodies known as "tetrazo-dyes," of which *Congo Red* is the type (Vol. 6), and which are remarkable for dyeing cotton without a mordant.

COMMERCIAL METHOD FOR THE ANALYSIS OF BENZIDINE (BASE). $NH_2.C_6H_4.C_6H_4.NH_2$ 4.4′ M. W. 184.17

1. *Moisture.*—Weigh a 2.0 grm. sample into an ignited, tared, low form, 50 c.c. porcelain crucible. Dry to constant weight *in vacuo* over conc. H_2SO_4 at room temperature. Loss in weight is moisture. Reserve the sample for the estimation of ash.

$$\frac{\text{Loss in weight} \times 100}{2} = \%\text{ moisture}$$

2. *Ash.*—Ignite the sample from 1 until free from carbonaceous matter, cool and weigh.

$$\frac{\text{Wt. of residue} \times 100}{2} = \%\text{ ash}$$

3. *Solubility.*—Place 5.0 grams of the finely pulverized sample in a beaker, add 250 c.c. boiling water and 25 c.c. conc. HCl acid. Boil for a few minutes, filter through a tared Gooch crucible, wash with hot water, dry and weigh.

$$\frac{\text{Wt. of residue} \times 100}{5} = \%\text{ insoluble}$$

4. *Base.*—Weigh 2.0 grm. of the powdered sample on a watch glass, wash into a beaker, add about 400 c.c. boiling water and 20 c.c. conc. HCl acid. Boil for a few minutes to insure solution, cool, transfer to a 500 c.c. volumetric flask and dilute to volume.

Pipette 100 c.c. of this solution into a 600 c.c. beaker, add 20 c.c. conc. HCl acid and sufficient water to make a volume of 300 c.c. Adjust the temperature to 20° and titrate with standard N/10 sodium nitrite solution until after standing for 10 minutes a faint reaction for excess nitrite is obtained with starch-iodide solution when spotted on a white porcelain spot plate.

$$\frac{\text{c.c. N/10 nitrite used} \times 0.0092085 \times 100}{0.4} = \%$$

Standard Nitrite Solution.—Dissolve 7.25 grm. of commercial, approximately 95% sodium nitrite per litre of water and standardise by titrating in the same manner at 15°–20° C. against recrystallised sulphanilic acid.

Determine the purity of the sulphanilic acid by a Kjeldahl nitrogen determination.

5. *Crystallising Point.*—Place portions of the sample in a 1″ × 4″ Pyrex test tube, heat over the flame until just melted and until the tube is nearly full. Place the tube through a thin short cork stopper in a Dewar bulb 1¼″ × 4″ internal diameter. Insert a standardised M. C. A. R-3 thermometer, range 70 to 160° C. and stir rapidly while holding the 76 mm. mark at the surface. Avoid rubbing the walls as much as possible. After some super-cooling, crystallisation will proceed, accompanied by a rise in temperature. Note the highest temperature obtained, and apply any correction for the thermometer found by standardisation while immersed to the 76 mm. mark. This temperature is taken as the crystallising point.

Orthotolidine. $NH_2.C_6H_3(CH_3).(CH_3)C_6H_3.NH_2$.—This base is homologous with benzidine, and is prepared from ortho-nitrotoluene by the same process by which benzidine is prepared from nitrobenzene. It melts at 128°, and presents a close resemblance to benzidine. The tetrazo-dyes prepared from it are less readily altered by acids than are the similar dyes prepared from benzidine.

According to Roesler and Glasmann, *Chem. Zeit.*, 1903, **27**, 986. Benzidine and Tolidine may be estimated iodometrically.

The method depends on the reaction of the bases with 1 molecule of iodine, according to the equations

$$C_{12}H_{12}N_2 + I_2 = C_{12}H_{11}IN_2 + HI$$
(Benzidine.)
$$C_{12}H_{16}N_2 + I_2 = C_{14}H_{15}IN_2 + HI$$
(Tolidine.)

About 5 grm. of the base are dissolved in 5 c.c. of hydrochloric acid, sp. gr. 1.19, and water, by the aid of heat, and when cold, diluted to 500 c.c. with water. 25 c.c. of this solution are neutralised with sodium bicarbonate solution until precipitation commences. This precipitate is redissolved in a trace of very dilute hydrochloric acid, care being taken that the final solution is perfectly neutral, since the presence of free acid will vitiate the results. The neutral solution thus obtained is diluted to 500 c.c., and titrated in the usual manner with N/20 iodine solution, run in drop by drop. The iodo-derivatives of the bases form voluminous dark blue precipitates, so that the end reaction is best observed either with starch paper or by spotting out with starch solution on a white tile.

COMMERCIAL METHOD FOR THE ANALYSIS[1] OF TOLIDINE (BASE). 4.3 $NH_2.CH_3.C_6H_3.C_6H_3,CH_3.$-$NH_2$ 3'.4' M. W. 212.21

1. *Solubility.*—Place 5 grm. of the finely pulverised sample in a beaker, add 250 c.c. boiling water and 25 c.c. conc. HCl. Boil for a few minutes, filter through a tared Gooch crucible, wash with hot water, dry and weigh.

$$\frac{\text{Wt. of residue} \times 100}{5} = \% \text{ insoluble}$$

2. *Base.*—Weigh 2 grm. of the powdered sample on a watch glass, wash into a beaker, add about 400 c.c. boiling water and 20 c.c. conc. HCl. Boil for a few minutes to insure solution, cool, transfer to a 500 c.c. volumetric flask and dilute to volume.

Pipette 100 c.c. of this solution into a 600 c.c. beaker, add 20 c.c. conc. HCl and sufficient water to make a volume of 300 c.c. Adjust the temperature to 20° and titrate with standard N/10 sodium nitrite solution until after standing for 10 minutes a faint reaction for excess nitrite is obtained with starch iodide solution when spotted on a white porcelain spot plate.

$$\frac{\text{c.c. N/10 nitrite used} \times 0.01061 \times 100}{0.4} = \%$$

Standard Nitrite Solution.—Dissolve 7.25 grm. of commercial, approximately 95% sodium nitrite per litre of water and standardise by titrating in the same manner at 15°–20° against recrystallised sulphanilic acid.

Estimate the purity of the sulphanilic acid by a Kjeldahl nitrogen determination.

3. *Crystallising Point.*—Place portions of the sample in a 1" × 4" Pyrex test tube, heat over the flame until just melted and until the tube is nearly full. Place the tube through a thin short cork stopper in a Dewar bulb 1¼" × 4" internal diameter. Insert a standardised M. C. A. R-3 thermometer, range 70 to 160°, and stir rapidly while holding the 76 mm. mark at the surface. Avoid rubbing the walls as much as possible. After some supercooling, crystallisation will proceed, accompanied by a rise in temperature. Note the highest temperature obtained, and apply any correction for

[1] The Newport Co., Carrollville Laboratories.

the thermometer found by standardisation while immersed to the 76 m.m. mark. This temperature is taken as the crystallising point.

4. *Sulphates.*—Weigh a 5 grm. sample, transfer to a beaker, add about 200 c.c. water and boil. Cool to room temperature, filter and wash with water. Heat the filtrate to boiling, add 5 c.c. conc. HCl and a few c.c. of hot $BaCl_2$ solution. Let stand on the steam bath for 1 hour and examine for precipitate of $BaSO_4$.

Dianisidine, 3-3′ dimethoxy-4-4′ diamino-diphenyl, $NH_2.OCH_2.$-$C_6H_5.C_6H_5.OCH_3.NH_2$. This homologue of benzidine as prepared by reducing ortho nitro-anisole with zinc dust and sodium hydroxide in the same manner as benzidine, but for high yields preferably in the absence of water. Its importance in the manufacture of some of the finest direct colours and relative high price make its examination in the laboratory a matter for careful consideration.

The base melts at 135° and is only sparingly soluble in water. It is sold almost entirely as the dry base in powder form. The powdered hydrochloride is highly irritant to the nasal passages and was used in shells in the late water, on account of its property of producing violent sneezing.

COMMERCIAL METHOD[1] FOR THE ANALYSIS OF DIANISIDINE. 4.3 $NH_2.OCH_3.C_6H_3.C_6H_3.OCH_3.NH_2$ 3′.4′ M. W. 244.21

1. *Moisture.*—Weigh 2 grm. sample into an ignited, tared, low-form, 50 c.c. porcelain crucible. Dry to constant weight in vacuo over conc. H_2SO_4 at room temperature. Loss in weight is moisture.

Reserve the sample for the estimation of ash.

$$\frac{\text{Loss in weight} \times 100}{2} = \%\text{ moisture.}$$

2. *Ash.*—Ignite the sample from 1 until free from carbonaceous matter, cool and weigh.

$$\frac{\text{Wt. of residue} \times 100}{2} = \%\text{ ash.}$$

3. *Solubility.*—Place 5 grm. of the finely pulverised sample in a beaker, add 250 c.c. boiling water and 25 c.c. conc. HCl. Boil for

[1] From the Newport Company—Carrollville Laboratory.

a few minutes, filter through a tared Gooch crucible, wash with hot water, dry and weigh.

$$\frac{\text{Wt. of residue} \times 100}{5} = \% \text{ insoluble.}$$

4. *Base.*—Weigh out 0.5 grm. of the finely pulverised sample on a watch glass, wash into a 600 c.c. beaker, add 400 c.c. boiling water and 25 c.c. conc. HCl. Boil for a few minutes to insure solution, cool to 20° and titrate with N/10 sodium nitrite solution until after standing for 10 min., a faint reaction for excess nitrite is obtained with starch iodide solution when spotted on a white porcelain spot plate.

$$\frac{\text{c.c. N/10 nitrite used} \times 0.01221 \times 100}{0.5} = \%$$

Standard Nitrite Solution.—Dissolve 7.25 grm. of commercial, approximately 95% sodium nitrite per litre of water, and standardise by titrating in the same manner at 15°–20° against recrystallised sulphanilic acid.

Determine the purity of the sulphanilic acid by a Kjeldahl Nitrogen determination.

5. *Crystallising Point.*—Place portions of the sample in a 1″ × 4″ Pyrex test tube, heat over the flame until just melted and until the tube is nearly full. Place the tube through a thin short cork stopper in a Dewar bulb 1¼″ × 4″ internal diameter. Insert a standardised M. C. A. R-3 thermometer, range −70 to 160° and stir rapidly while holding the 76 m.m. mark at the surface. Avoid rubbing the walls as much as possible. After some super-cooling, crystallisation will proceed, accompanied by a rise in temperature. Note the highest temperature obtained and apply any correction for the thermometer found by standardisation while immersed to the 76 mm. mark. This temperature is taken as the crystallising point.

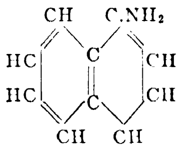
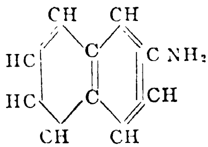
The analyst is referred to Thorpe's Dictionary of Applied Chemistry, which includes the best articles in English on all of these subjects, and to Teerfarbenfabrikation and verwandter Industriezweige by Friedlaender, if he wishes to go to the patents.

NAPHTHYLAMINES, PYRIDINE, QUINOLINE AND ACRIDINE BASES

BY A. B. DAVIS

NAPHTHYLAMINES AND THEIR ALLIES

When naphthalene, $C_{10}H_8$, is treated cautiously with nitric acid, α-nitronaphthalene, $C_{10}H_7NO_2$, is formed, and this is converted by reducing agents into α-aminonaphthalene or α-naphthylamine, $C_{10}H_7.NH_2$. By other reactions the isomeric β-naphthylamine may be obtained. These two substances differ from each other in a notable manner, as indicated in the following table:

	α-Naphthylamine	β-Naphthylamine
Structural formula...........		
M. p.	50°	112°
B. p....	300°	294°
Odour......	Disagreeable; persistent.	None.
Appearance	Flat needles or prisms.	Pearly plates.
Reactions of hydrochloride in solution: With ferric chloride..........	Blue precipitate.	No reaction.
With nitrous acid in alcoholic or acetic acid solution.	Yellow colour, turned crimson by hydrochloric acid.	No reaction.
With sulphanilic acid and sodium nitrite, followed by hydrochloric acid.	Red coloration.	....

α-Naphthylamine. $C_{10}H_7.NH_2$

This base is obtained by the reduction of nitronaphthalene, or by heating α-naphthol with the double compound of calcium chloride and ammonia.[1]

[1] On a large scale, α-naphthylamine is prepared in a manner very similar to that employed for the production of aniline. Nitronaphthalene is reduced by iron and hydrochloric

α-Naphthylamine has a most disgusting and persistent odour, resembling that of fæces. It turns violet or brown in the air, but when purified by sublimation this change occurs very slowly, and only on exposure to air and light. It is slightly volatile with steam.

α-Naphthylamine is nearly insoluble in water, but very soluble in alcohol and ether. It forms a series of readily-crystallisable, easily-soluble salts; the *hydrochloride*, $C_{10}H_7.NH_2,HCl$, forms long needles and glistening scales, sublimes at 200°, and is soluble in water, ethyl alcohol and ether; the *sulphate* (tech. *Naphthylamine* S), $(C_{10}H_7NH_2)_2,H_2SO_4$, crystallises with $2H_2O$ in white, silvery scales and is soluble with difficulty in cold water and ethyl alcohol, but readily soluble in hot ethyl alcohol; the *oxalate*, $(C_{10}H_7.NH_2)_2,H_2C_2O_4$, forms small, glistening leaflets; the *hydrogen oxalate*, $C_{10}H_7.NH_2,H_2C_2O_4$, crystallises in white nodules; the *picrate* has m. p. 165°. When ammonia is added to a solution of a salt, the free base is precipitated in white, silky needles.

On adding ferric chloride to a solution of α-naphthylamine, or of one of its salts, an azure blue precipitate of *naphthameïn* is produced, which rapidly becomes purple, but is unchanged by treatment with sulphurous acid. Other oxidising agents (*e. g.*, chromic acid, bleaching powder) produce precipitates varying in colour from blue to violet or red.

On adding an alcoholic solution of nitrous acid to a solution of α-naphthylamine in alcohol or glacial acetic acid, a yellow colour is produced, which, on adding a little hydrochloric acid, changes to an intense violet or magenta colour; or, in presence of only traces of naphthylamine, to a reddish colour.

A red coloration is produced when hydrochloric acid is added to a cold solution of α-naphthylamine, sulphanilic acid and sodium nitrite, owing to the formation of aminonaphthylazobenzenesulphonic acid, $NH_2.C_{10}H_6.N_2.C_6H_4.SO_3H$.

Commercial α-naphthylamine is met with in white, grey or brown crystalline cakes; it should be free from oily constituents and almost completely soluble in dilute hydrochloric acid. *Naphthalene*, the presence of which causes incomplete solubility, may be estimated by

acid at a temperature of about 50°. When the reduction is complete, milk of lime is added and the naphthylamine distilled off by the aid of superheated steam. The crude product is purified by redistillation, when it is obtained as a nearly colourless oil, which solidifies to crystalline cakes of a greyish colour. It appears to be wholly free from β-naphthylamine, but contains an impurity which is probably 1:8-naphthylenediamine, $C_{10}H_6(NH_2)_2$ (Witt, *Dingl. polyt. Jour.*, 1887, **265**, 225).

distilling the acidified solution in a current of steam, agitating the distillate with ether, separating the ethereal layer, evaporating at a low temperature and weighing the residue. The technical product may also contain some *β-naphthylamine;* this is isolated by the fractional crystallisation of the hydrochloride and, subsequently, of the sulphate.

α-Naphthylamine, when boiled with glacial acetic acid, yields *α-acetnaphthalide*, $C_{10}H_7.NHAc$, m. p. 159–160°, and when treated with benzoyl chloride and aqueous sodium hydroxide yields *benz-α-naphthalide* (benzoyl-α-naphthylamine), $C_{10}H_7.NHBz$, m. p. 156°. α-Naphthylamine is used in the preparation of α-naphthol, α-naphthylamine sulphonic acids, azo-dyes, Magdala Red (Vol. 6), and naphthalene fancy colours for cotton.

β-Naphthylamine. $C_{10}H_7.NH_2$

This modification of aminonaphthalene is most readily obtained by heating β-naphthol under pressure with ammonia at 160°, or with the double compound of zinc chloride and ammonia at 200–210°. Sodium bisulphite and ammonia are much used for large scale production.

β-Naphthylamine is odourless and more stable than the α-modification. It volatilises in a current of steam, and is slightly soluble in cold, more readily in hot water, the solution exhibiting a blue fluorescence, which, however, is not shown by β-naphthylamine salts. β-Naphthylamine gives no coloration with oxidising agents, nor with nitrous and hydrochloric acids in alcoholic solution.

The *hydrochloride*, $C_{10}H_7.NH_2, HCl$, crystallises in leaflets and is readily soluble in water and ethyl alcohol; the *sulphate*, $(C_{10}H_7NH_2)_2,H_2SO_4$, crystallises in leaflets and is less soluble in water than the α-salt; the *picrate* crystallises in long, yellow needles, m. p. 195° (decomp.), and is soluble in alcohol.

β-Naphthylamine, when heated with (1) formic acid (sp. gr. 1.2), yields *formyl-β-naphthalide*, $C_{10}H_7.NH.CHO$, m. p. 129°, and (2) glacial acetic acid yields *β-acetnaphthalide*, crystallising in white leaflets, m. p. 132°.

Commercial β-naphthylamine should have m. p. 112° and be almost completely soluble in dilute hydrochloric acid; the insoluble portion is probably *β-naphthol* and *β-dinaphthylamine;* the β-naphthol is

isolated by extracting the product with dilute aqueous potassium hydroxide and acidifying the alkaline extract with a mineral acid.

β-Naphthylamine is used in the preparation of red azo-dyes.

Tetrahydro-β-naphthylamine. $C_{10}H_{11}.NH_2$

This base has been introduced into medicine under the name of "Thermine." It is a colourless, slightly viscous liquid, of peculiar odour. It is a strong base, a drop soon becoming converted into a crystalline mass of the *carbonate* on exposure to air. The *hydrochloride* forms well-defined white crystals, m. p. 237°, and is readily soluble in water, alcohol, and amyl alcohol.

The physiological effects of Thermine embrace the two strongly marked characteristics of mydriasis (accompanied by pain) and elevation of the temperature, which latter effect has been observed to the extent of 4.5°.

ALKYL- AND ACYL-NAPHTHYLAMINES

Ethyl-α-naphthylamine, $C_{10}H_7.NHEt$, when freshly distilled, is a colourless oil, but on exposure to the air it becomes brownish-red by transmitted light and steel-blue by reflected light; it has sp. gr. at 18°/18°, 1.073.

Ethyl-β-naphthylamine, when freshly distilled, is a colourless, viscid oil, b. p. 315–316°; 305°/716 mm. 191°/25 mm., sp. gr. at 18° 1.062, soluble with difficulty in hydrochloric acid. It comes on the market as *Developer* B and its use has been proposed for the preparation of black azo-dyes.

A method for estimating the above two substances is given by Vaubel (*Chem. Zeit.*, 1903, **27**, 278); reliable results are obtained, however, only by an experienced worker.

Phenyl-α-naphthylamine, $C_{10}H_7.NHPh$, is prepared by heating aniline with α-naphthylamine hydrochloride or α-naphthylamine with aniline hydrochloride at 240°; it crystallises in prisms, m. p. 62°, b. p. 226°/15 mm., 335°/258 mm., is insoluble in dilute acids, and easily soluble in most organic solvents. The *acetyl* derivative has m. p. 115°; the *benzoyl* derivative has m. p. 152°. The *commercial* product is usually met with as pale brown cakes; it is used in the preparation of Victoria Blue.

Phenyl-β-naphthylamine is obtained by heating β-naphthol, aniline and aniline hydrochloride at 200–210°; it crystallises in

needles, m. p. 108°, b. p. 395°, is slightly soluble in cold alcohol, ether, benzene and glacial acetic acid, and readily soluble in the hot solvents forming solutions with a blue fluorescence. The *hydrochloride*, formed by passing hydrogen chloride into a solution in benzene, is decomposed by water; the *acetyl* derivative has m. p. 93°; the *benzoyl* derivative has m. p. 136°.

o-**Tolyl-α-naphthylamine,** $C_{10}H_7.NH.C_6H_4.Me$, is prepared by heating α-naphthol and *o*-toluidine with calcium chloride at 280°; it crystallises in needles, m. p. 94–95°, is slightly soluble in light petroleum, readily soluble in the ordinary organic solvents. The addition of nitric acid to the greenish-yellow solution in sulphuric acid produces a dark greenish-blue colour changing to yellowish-brown, whilst potassium dichromate, in small quantity, gives a dirty dark-green, in larger quantity, a reddish-brown coloration.

o-**Tolyl-β-naphthylamine,** prepared from β-naphthol, *o*-toluidine and calcium chloride, crystallises in glistening leaflets, m. p. 95–96°; it is decomposed by concentrated hydrochloric acid yielding *o*-toluidine and β-naphthol. The pale yellow solution of the base is concentrated sulphuric acid is coloured dark reddish-yellow by nitric acid, and brownish-violet by potassium dichromate. The *picrate* has m. p. 110°; the *benzoyl* derivative has m. p. 117–118°.

p-**Tolyl-α-naphthylamine** is obtained by heating α-naphthylamine with *p*-toluidine hydrochloride or α-naphthol and *p*-toluidine with calcium chloride; it crystallises in short prisms, m. p. 79°; is slightly soluble in cold alcohol and light petroleum, readily soluble in boiling alcohol, ether and benzene. The pale yellow solution in concentrated sulphuric acid is coloured green by the addition of nitric acid or potassium dichromate. It is used in the preparation of Night Blue.

p-**Tolyl-β-naphthylamine** is prepared by heating β-naphthol, *p*-toluidine, and *p*-toluidine hydrochloride at 200–210°; it forms reddish leaflets, m. p. 102–103°, is soluble in hot organic solvents, and slightly soluble in cold alcohol and light petroleum. The yellow solution in concentrated sulphuric acid gives a brownish-red colour with nitric acid and a raspberry-red colour with potassium dichromate. It is decomposed by concentrated hydrochloric acid, yielding *p*-toluidine and β-naphthol. The *acetyl* derivative forms colourless needles, m. p. 85°; the *benzoyl* derivative crystallises in needles,

m. p. 139°. The base finds application in the preparation of Wool Black.

Benzyl-α-naphthylamine, $C_{10}H_7.NH.CH_2Ph$, is prepared from α-naphthylamine and benzyl chloride; it has m. p. 66–67° and is used in the preparation of Nile Blue B.B.

Dimethyl-α-naphthylamine, $C_{10}H_7.NMe_2$, is prepared by heating α-naphthylamine with methyl iodide and methyl alcohol at 100° or α-naphthylamine hydrochloride with methyl alcohol at 180°; it is a liquid with a green fluorescence, b. p. 267°, and has an odour resembling petroleum.

Dimethyl-β-naphthylamine, is obtained by the action of dimethylamine on β-naphthol at 200°; it forms crystals, m. p. 46°, b. p. 305°.

Diethyl-α-naphthylamine, $C_{10}H_7,NEt_2$, is formed by heating α-naphthylamine with ethyl bromide and alcohol under pressure at 120°; it is a yellow oil, b. p. 290°.

Methylphenyl-α-naphthylamine, $C_{10}H_7.NMePh$, is prepared by acting on phenyl-α-naphthylamine and methyl alcohol with hydrogen chloride under pressure; it is a greenish oil with a blue fluorescence, and is employed in the preparation of Victoria Blue.

α-Dinaphthylamine, $(C_{10}H_7)_2NH$, is prepared by heating α-naphthylamine with its hydrochloride at 150°. It crystallises in large rectangular leaflets, m. p. 113°, and is insoluble in water, moderately soluble in alcohol, readily soluble in the other organic solvents. The solution in concentrated sulphuric acid is yellow at first, but quickly, turns green, especially when warmed. An alcoholic solution gives a pale green colouration with ferric chloride. The base is not altered by acetic anhydride, but with acetyl chloride it yields an *acetyl* derivative which crystallises in needles, m. p. 217°. The *picrate*, $(C_{10}H_7)_2NH$, $2C_6H_3O_7N_3$, forms needles, m. p. 168–169°.

αβ-Dinaphthylamine, $C_{10}H_7[\alpha]NH[\beta]C_{10}H_7$, is prepared by heating α-naphthylamine and β-naphthol with crystalline calcium chloride at 280°; it crystallises in long, stout, glistening prisms, m. p. 110–111°, and is readily soluble in warm alcohol, ether and benzene; the *picrate* crystallises in needles, m. p. 172–173°; the *acetyl* derivative forms stout needles, m. p. 125°.

β-Dinaphthylamine is formed by heating β-naphthol with zinc ammonium chloride under pressure at 280–300°; it crystallises in silvery leaflets, m. p. 170.5°, is slightly soluble in alcohol, readily in

hot glacial acetic acid and benzene; the solutions exhibit a blue fluorescence. The *picrate* has m. p. 164–165°; the *acetyl* derivative crystallises in needles, m. p. 114–115°.

Naphthylene-Diamines. $C_{10}H_6(NH_2)_2$

The naphthylenediamines or diaminonaphthalenes are prepared by heating the corresponding dihydroxynaphthalenes with ammonia, by the reduction of the dinitronaphthalenes, and in other ways.

The table on page 616 exhibits the leading properties of the most important members which find application in the dyeing industry.

Naphthylaminesulphonic Acids.—It is not possible in a work of this kind to give a complete account of all the naphthylaminesulphonic acids known, but in the following pages the properties of the most important technical products are briefly summarised. The general methods of preparation are also mentioned, as this gives an indication of the impurities to be looked for in testing the commercial material.

General Methods of Preparation.

1. By sulphonation at different temperatures of the naphthylamines.

2. By sulphonation of the nitronaphthalenes and reduction of the product.

3. By nitration of the naphthalenesulphonic acids and reduction of the product.

4. By the action of ammonia on the naphtholsulphonic acid, the OH group being replaced by the NH_2 group.

Monosulphonic Acids of α-Naphthylamine

Most of the naphthylaminemonosulphonic acids are commercially important, chiefly in connection with the manufacture of the dyes which are obtained by combining them with diazo-compounds.

1-Naphthylamine-2-sulphonic acid (*ortho-naphthionic acid*) is prepared by heating sodium naphthionate with naphthalene at 200°, the SO_3H group changing from position 4 to 2; it crystallises in triclinic needles, 1 part dissolving in 225 parts of water at the ordinary temperature; reduces solutions of gold salts; gives a greyish-green precipitate with ferric chloride and yields a *diazo*-compound which forms difficultly soluble greenish-yellow plates. **Salts:** *sodium*,

THE NAPHTHALENE DIAMINES

Position of the amino groups	1 : 2-	1 : 3-	1 : 4-	1 : 5-	1 : 8-	2 : 3-	2 : 7-
Mode of preparation.	By reducing azo-compounds of β-naphthylamine.	From 1 : 3-dinitronaphthalene and from 3-amino-α-naphthol.	From 4-nitro-α-naphthylamine and from azo-compounds of α-naphthylamine.	From 1 : 5-dinitronaphthalene.	From 1 : 8-dinitronaphthalene.	From 2 : 3-dihydroxynaphthalene.	From 2 : 7-dihydroxynaphthalene.
Form of crystals.	Plates.	Leaflets.	Leaves.	Prisms.	Needles.	Leaves.	Leaflets.
M. p.	95°	96°	120°	189.5°	66.5°	191°	159°
Hydrochloride.	Short prisms.	Needles.	Plates.	Needles.	Leaves, m. p. 280°.	....	....
Sulphate.	Leaflets.	Needles.	....	Needles.	Small crystals.	....	....
Reaction of the hydrochloride with ferric chloride.	Green, then yellow coloration, brown precipitate.	Dark-brown coloration.	Green coloration.	Blue coloration then blue precipitate.	Chestnut-brown precipitate.	No coloration.	....
Action of nitrous acid.	....	Deep yellow coloration.	Soluble tetrazo-compound.	Soluble tetrazo-compound.	Vermilion precipitate.	Yields naphthylene-azo-imide, $C_{10}H_6N_3H$, yellow needles, m. p. 187°.	....
Action of the azo-dyestuffs on unmordanted cotton.	....	....	Do not dye.	Dye the fibre.	....	....	....
Diacetyl derivative.	Needles, m. p. 234°.	Slender white needles, m. p. 363°.	Crystals, m. p. 303–304°.	....	....	Brown feathery needles, m. p. 247°.	Granules, m. p. 197.5°.

difficultly soluble, 1 part dissolving in 60 parts of cold water or 10 parts of boiling water; aqueous solution exhibits dark green fluorescence; *calcium*, very difficultly soluble large, flat prisms, 1 part dissolving in 20 parts of water at 100°.

1-Naphthylamine-3-sulphonic acid (γ) is formed in small quantities in the preparation of 1:6- and 1:7-naphthylaminesulphonic acids by nitration and subsequent reduction of 2-naphthalenesulphonic acid; it crystallises in colourless needles, soluble with difficulty. **Salts:** *sodium*, easily soluble; *barium* ($1H_2O$), flat, glistening needles.

1-Naphthylamine-4-sulphonic acid (*naphthionic acid*) is prepared by baking α-naphthylamine hydrogen sulphate at 170–180° with 3% of oxalic acid; it is soluble with difficulty in cold water, 1 part dissolving in 4000 parts of water at 15°, and crystallises with $\frac{1}{2}H_2O$; ferric chloride colours the aqueous solution brown, which darkens on boiling. The acid, when heated with aqueous solutions of alkalies under pressure at 200–250°, yields the corresponding 1-naphthol-4-sulphonic acid. Concentrated sulphuric acid gives rise to disulphonic acid derivatives, whilst dilute sulphuric acid decomposes it into α-naphthylamine and sulphuric acid. **Salts:** *sodium* ($4H_2O$), large, monoclinic, colourless crystals, easily soluble in water, insoluble in alcohol precipitated from aqueous solutions by sodium hydroxide and salt; the solutions show an intense reddish-blue fluorescence; the solid salt, when shaken with benzaldehyde, yields the *benzylidene* derivative, $CHPh{:}N.C_{10}H_6.SO_3Na$, which crystallises in golden yellow plates and is decomposed by acids; *calcium* ($8H_2O$), characteristic crystals, soluble in water.

1-Naphthylamine-5-sulphonic acid (naphthalidinic acid; Laurent's or L-acid) is prepared by sulphonating α-naphthylamine with concentrated sulphuric acid at 130°. Naphthionic acid is the initial product, but on prolonged heating the 1:5- and 1:6-acids alone are formed. It crystallises from hot aqueous solutions with $1H_2O$ in small plates, 1 part dissolving in 950 parts of water at 15°, and when fused with sodium hydroxide, yields 1-naphthol-5-sulphonic acid. **Salts:** *sodium* ($1H_2O$), small, very soluble prisms; *calcium* ($9H_2O$), triangular plates, readily soluble in water; shaken with benzaldehyde it gives difficultly soluble needles of the *benzylidene* compound.

Aqueous and alcoholic solutions of the acid and its salts show a green fluorescence.

1-Naphthylamine-6-sulphonic acid (β) (Cleve's β-acid), produced with the 1:7- and 1:3- acids when naphthalenesulphonic acid is nitrated and subsequently reduced, crystallises in needles and plates according to the solvent used; 1 part of the acid dissolves in 1000 parts of water at 15°, or 100–200 parts of water at 100°; aqueous solutions give with ferric chloride or gold chloride a cornflower-blue or reddish-violet coloration respectively; it is very stable and is not decomposed by dilute acids at 200°. **Salts:** *sodium* ($4H_2O$), rhombic plates; *calcium* ($7H_2O$), slow crystallisation from hot concentrated solutions ($2H_2O$).

1-Naphthylamine-7-sulphonic acid (Cleve's J-acid) crystallises in needles or flat prisms, and dissolves in 215 parts of water at 25°; ferric chloride gives an intense blue coloration, which on the addition of acetic acid becomes red. **Salts:** *sodium* ($\frac{1}{2}H_2O$), thin needles, readily soluble in water and methyl alcohol; *zinc* ($4H_2O$), glistening yellow needles, soluble with difficulty in water. This and the former acid are combined with diazo-compounds to produce black azo-dyes for cotton fabrics.

1-Naphthylamine-8-sulphonic acid (Schöllkopf's or S acid) is prepared by cold nitration and subsequent reduction of naphthalenesulphonic acid, the 1:5-acid being formed simultaneously; it crystallises in white needles, soluble in 4800 parts of water at 21° or 240 parts at 100°. Ferric chloride colours the cold saturated solution an intense violet, whilst gold chloride gives a red coloration changing to violet and violet fluorescence. The acid does not condense with benzaldehyde; the *diazo*-compound forms difficultly soluble greenish-yellow prisms. **Salts:** *sodium* plates, 1 part dissolves in 88.5 parts of water at 24° or 37.5 parts at 100°; *potassium*, large, shining plates, 1 part dissolves in 28 parts of water at 19° or 6.7 parts at 100°.

The salts of this acid have the peculiar property of clinging tenaciously to ferric and aluminium oxides.

Monosulphonic Acids of β-Naphthylamine

The most general method of preparing these acids is by heating the corresponding β-naphtholsulphonic acids with strong aqueous ammonia under pressure.

Most of the β-naphthylaminesulphonic acids are of importance.

2-Naphthylamine-5-sulphonic acid (*Dahl's acid*) crystallises in long, slender needles, soluble in 1000 parts of cold or 200 parts of hot water; the *diazo*-compound forms greyish-yellow, difficultly soluble crystals. **Salts:** *sodium* ($5H_2O$), large, thick plates, very soluble in water; *potassium* ($1H_2O$), small rhombohedra, very soluble in water.

Aqueous solutions of the acid and salts exhibit a reddish-blue fluorescence.

2-Naphthylamine-6-sulphonic acid (*Brönner's* or *β-acid*) forms long, silky, prismatic needles, soluble in 630 parts of water at 100°; the *diazo*-compound is a difficultly soluble greenish-yellow powder. **Salts:** *sodium* ($2H_2O$), white, silky needles, 1 part soluble in 40 parts of water; *calcium* ($6H_2O$), long silky needles, 1 part dissolves in 225 parts of cold water; *ammonium* ($1H_2O$), silky needles or plates with a violet fluorescence.

Aqueous solutions of the acid and salts exhibit a blue fluorescence.

2-Naphthylamine-7-sulphonic acid (*F*- or δ-acid, *Baeyer's acid, Casella's acid* F) forms long, silky needles almost insoluble in cold water, soluble in 350 parts of hot water; the *diazo*-compound forms crystals which appear orange-red by reflected light and colourless by transmitted light. **Salts:** *sodium* ($4H_2O$), needles, difficultly soluble in cold, more easily in hot, water; *ammonium*, small, soluble plates; *calcium* ($6H_2O$), blue fluorescent plates. 1 part dissolves in 280 parts of cold water; *magnesium* ($5\frac{1}{2}$ $2H_2O$), small, blue fluorescent needles.

Aqueous solutions of the acid and salts exhibit a reddish-violet fluorescence.

2-Naphthylamine-8-sulphonic acid (*α-acid* or *Baden acid*) forms small prisms, 1 part soluble in 1700 parts of cold water and almost insoluble in alcohol. **Salts:** *sodium*, glistening tablets, insoluble in alcohol; *potassium* ($\frac{1}{2}H_2O$), six-sided plates; *calcium*($6H_2O$), large, thick plates. 1 part dissolves in 11 parts of water.

Aqueous solutions of the acid and salts exhibit a pale blue fluorescence.

The β-naphthylaminesulphonic acids give characteristic coloured compounds with benzidine and tolidine, which are very useful in characterising the different isomeric acids. The coloured substances obtained with the three most important of the acids are shown in the following table.

Acid $NH_2:SO_3H$	Colours of the	
	Benzidine compound	Tolidine compound
2:5-		Yellow.
2:6-	Yellowish-red	Yellowish-red (Benzopurpurin 1 B).
2:7-		Bluish-red; insoluble magnesium salt; completely precipitated by acetic acid.

Disulphonic Acids of α-Naphthylamine

1-Naphthylamine-3:6-disulphonic acid (*Freund's acid*), prepared by reducing 1-nitronaphthalene-3:6-disulphonic acid, is readily soluble in water and alcohol, insoluble in ether and benzene; when heated with water it yields α-naphthol-3:6-disulphonic acid.

1-Naphthylamine-3:8-disulphonic acid (*ξ-naphthylaminedisulphonic acid*), prepared by nitration and subsequent reduction of 1:6-naphthalenedisulphonic acid, crystallises in colourless, glistening scales ($3H_2O$), and is extremely soluble in water; the *diazo*-compound forms white needles. **Salts**: *sodium hydrogen* ($2H_2O$), long needles, 1 part dissolves in 30 parts of cold water; *disodium* ($6H_2O$), easily soluble in water; *copper*, flesh-coloured powder almost insoluble in water.

Aqueous solutions of the acid and salts have a green fluorescence.

1-Naphthylamine-4:6-disulphonic acid (*Dahl's acid* No. II). When naphthionic acid is sulphonated, 33% of this acid and 66% of 1-naphthylamine-4:7-disulphonic acid are produced; it is readily soluble in hot water, insoluble in alcohol; ferric chloride produces a white turbidity, and gold chloride gives an orange coloration; the *diazo*-compound forms silky, yellow needles. **Salts**: *calcium hydrogen*, readily soluble white needles, forms double salts with other bases; *calcium*, glistening needles, soluble in hot methyl alcohol, in 85% alcohol, but not in absolute alcohol.

Aqueous solutions of the acid and salts exhibit a blue fluorescence.

1-Naphthylamine-4:7-disulphonic acid (*Dahl's acid* No. III) crystallises in rosettes of needles, soluble in 20 parts of hot, sparingly soluble in cold, water, insoluble in 85% alcohol; the *diazo*-compound is a yellow, amorphous powder, difficultly soluble in water.

Aqueous solutions of the acid and salts exhibit a blue fluorescence.

1-Naphthylamine-4:8-disulphonic acid (δ- or S-*acid*) is prepared by heating α-naphthylaminemonosulphonic acid S with fuming sulphuric acid at 100° till the product is soluble in water; it crystallises in readily soluble rhombohedra. **Salts:** *sodium hydrogen*, difficultly soluble long prisms; *sodium*, colourless needles or ($2H_2O$) compact clear crystals.

Aqueous solutions of the acid and salts exhibit a green fluorescence.

Disulphonic Acids of β-Naphthylamine

These acids are obtained by heating the corresponding naphtholsulphonic acids with aqueous ammonia.

2-Naphthylamine-3:7-disulphonic acid (δ-*naphthylaminedisulphonic acid*) can be prepared by the reduction of 2-nitronaphthalene-3:7-disulphonic acid; it is sparingly soluble in cold, readily in hot water; the *sodium* salt ($1H_2O$) forms small needles, soluble in 50 parts of water at the ordinary temperature.

2-Naphthylamine-6:8-disulphonic acid, (*amido*-G-*acid*), prepared by the action of fuming sulphuric acid on β-naphthylamine at 110–140°, is readily soluble in water and when fused with sodium hydroxide yields aminonaphtholmonosulphonic acid; the *diazo*-compound is readily soluble in water; the *calcium* and *barium* salts (normal) are readily soluble in water.

The acid is used in the form of its diazo-compound in the production of wool-blacks for dyeing.

Naphthylaminetrisulphonic Acids

1-Naphthylamine-3:6:8-trisulphonic acid is prepared by the sulphonation and nitration and subsequent reduction of naphthalene-β-sulphonic acid. The solutions in alkalies are not fluorescent. **Salts:** *disodium*, easily soluble in water, difficultly soluble in brine; *sodium hydrogen*, fine white needles.

2-Naphthylamine-3:6:8-trisulphonic acid is prepared by sulphonating β-naphthylaminesulphonic acid (G) with sulphuric acid, containing 40% of sulphur trioxide, at 120°; it is readily soluble in water; the *diazo*-compound forms yellow needles; the *azo*-compound with R salt is red and easily soluble; the *potassium hydrogen* salt forms sparingly soluble, glistening needles. Aqueous solutions of the acid and salts exhibit an intense sky-blue fluorescence.

Diaminonaphthalenesulphonic Acids

A few diaminonaphthalenesulphonic acids have come into use for the manufacture of dyestuffs, and therefore are of some importance.

1:4-Diaminonaphthalene-6-sulphonic acid, prepared by the nitration and subsequent reduction of 1:5- or 1:7-naphthylamine-monosulphonic acid, the first amino group being protected by acetylation, is a crystalline powder readily soluble in water.

1:5-Diaminonaphthalene-3:7-disulphonic acid, similarly prepared from naphthalene-2:6-disulphonic acid, forms microscopic crystals, insoluble in water; the *sodium* salt is soluble in 20 parts of cold water.

1:8-Diaminonaphthalene-3:6-disulphonic acid, prepared by the reduction of 1:8-dinitronaphthalene-3:6 disulphonic acid with ammonium sulphide, is slightly soluble in water; ferric chloride colours an aqueous solution reddish-brown. **Salts:** *potassium* ($3H_2O$), thin needles, fairly soluble in hot water; *sodium*, slender needles; *barium hydrogen*, slender, glistening needles, difficultly soluble in hot water; *barium* ($6H_2O$), slender needles difficultly soluble in hot water.

Aqueous solutions of the acid and salts are not fluorescent.

Analysis of Naphthylaminesulphonic Acids

Various methods have been proposed for the analysis of the naphthylaminesulphonic acids, and used with more or less success.

Two methods much in use are the titration of the free acid or its sodium salts with a standard solution of 1. sodium nitrite or 2. diazobenzene or diazotoluene. The dye formed is salted out, and the end-point ascertained by taking a drop of the solution on a filter paper and adding a drop of the diazo-solution to see if any more dye is formed. Even with the most skilful workers the degree of accuracy is not greater than 1–2%.

Another method proposed by Vaubel (*Chem. Zeit.*, 1893, **17**, 1265) depends on the absorption of bromine by some of the sulphonic acids. He finds that they can be divided into 3 classes:

1. Sulphonic acids which absorb 1 atom of bromine.
2. Sulphonic acids which slowly absorb several atoms of bromine.
3. Sulphonic acids which do not absorb bromine at the ordinary temperature.

Class 1 includes the following acids:

1-Naphthylamine-2-sulphonic acid.
1-Naphthylamine-4-sulphonic acid.
1-Naphthylamine-4:6-disulphonic acid.
1-Naphthylamine-4:7-disulphonic acid.
1-Naphthylamine-4:8-disulphonic acid.
2-Naphthylamine-3:6-disulphonic acid.
2-Naphthylamine-5-sulphonic acid (2 Br.).
1:6-Diaminonaphthalene-4-sulphonic acid.

Class 2 includes:

1-Naphthylamine-7-sulphonic acid which absorbs 3 Br.
1-Naphthylamine-8-sulphonic acid which absorbs 2 Br.
1-Naphthylamine-3:7-disulphonic acid which absorbs 2 Br.
2-Naphthylamine-6-sulphonic acid which absorbs 3 Br.
2-Naphthylamine-7-sulphonic acid which absorbs 3 Br.

Class 3 includes:

2-Naphthylamine-8-sulphonic acid.
2-Naphthylamine-6:8-disulphonic acid.

The method of procedure is to add to a weighed quantity of the sulphonic acid an excess of potassium bromide and sulphuric acid, and then a standard solution of potassium bromate until there is an excess of bromine; the estimation is carried out at the ordinary temperature.

In the case of those acids which fall under Class 3, it has been found that 1 atom of bromine is taken up at 65–75° without much loss of bromine; care must be taken, however, to keep the temperature below 75°, otherwise the end-point is not easily determined.

Aminonaphthols. $NH_2.C_{10}H_6.OH$

These substances are unstable bases obtained by the action of reducing agents on the nitro- or nitroso-naphthols, or on certain azo-dyes. The following table shows the leading differences of the principal members of the group:

	4-Amino-1-naphthol	2-Amino-1-naphthol	1-Amino-2-naphthol
Mode of formation.	Reduction of 4-nitro-α-naphthol; or of Orange I, (Vol. 6).	Reduction of 2-nitro-α-naphthol; or of nitroso-α-naphthol.	Reduction of 1-nitro-β-naphthol; of nitroso-β-naphthol; or of Orange II, (Vol. 6).
Characters of free base.	Unstable.	Needles from water containing sulphur dioxide; soluble with difficulty in water.	Colourless scales; slightly soluble in water; oxidised in the air. Ethereal solution exhibits violet fluorescence.
Reaction on agitating alkaline solution with air.	Dirty green coloration, changing to yellow.	Permanent grass-green colour, and green scum soluble in alcohol to pure green solution. Or violet naphthaquinonimide— $C_{10}H_6\left\{\begin{matrix}NH\\O\end{matrix}\right\}$	Brown coloration.
Reaction with bromine water.	Yellowish-white needles precipitated, even in very dilute solutions.	Yellowish or green precipitate (the same with ferric chloride).	. . . .
Characters of hydrochloride.	Long white needles or acicular plates. With bleaching powder yields $C_{20}H_{12}N_3Cl$, which separates from acetic acid solution in needles, melting at 85° and exploding at 130°.	White laminæ.	White lustrous needles; readily soluble in water, but only sparingly in dilute hydrochloric acid.
Product of oxidation with chromic acid mixture.	Theoretical yield of α-naphthaquinone.	β-naphthaquinone.	β-naphthaquinone.

Aminonaphtholsulphonic Acids

The aminonaphtholsulphonic acids are not so important as the naphthylaminesulphonic acids, but many of them have found useful application in the manufacture of dyes.

1-Amino-2-naphthol-6-sulphonic acid is prepared by the reduction of nitrosonaphtholsulphonic acid with tin and hydrochloric acid; it forms long, greyish-white needles, soluble with difficulty in boiling water and ethyl alcohol, insoluble in ether; with diazobenzenesulphonic acid it gives a fuschine red *dye*. **Salts**: sodium ($2\frac{1}{2}H_2O$), used as a photographic developer under the name of "*Eikonogen*" (R. Meldola, *J. Soc. Chem. Ind.*, 1889, **8**, 958).

Aqueous solutions of the acid and salts oxidise in air, turning brown.

1-Amino-2-naphthol-4-sulphonic acid forms bright yellow needles, insoluble in water, alcohol, ether, and benzene; it is decomposed by

concentrated hydrochloric acid at 150° with elimination of sulphuric acid, and in alkaline solution is oxidised by the air giving a brown *dye*, which dissolves in hot water, with a green colour.

1-Amino-8-naphthol-4-sulphonic acid (S-*acid*), prepared by fusing 1-naphthylaminedisulphonic acid (S) with potassium hydroxide, forms needles readily soluble in water, and combines with 2 molecules of a diazo-compound, yielding dark coloured *dyes;* ferric chloride gives an emerald-green coloration; alkaline solutions have an intense blue fluorescence.

2-Amino-1-naphthol-4-sulphonic acid, prepared by the reduction of the corresponding nitrosonaphtholsulphonic acid, forms pearly needles and plates ($1H_2O$), and when warmed with aqueous alkali in the presence of air yields a violet-black *dye.*

2-Amino-8-naphthol-6-sulphonic acid (G- or γ-*acid*), prepared by fusing β-naphthylaminedisulphonic acid with sodium hydroxide in an autoclave, forms white needles difficultly soluble in water, and reduces ammoniacal solutions of copper and silver salts; the *diazo*-compound is a difficultly soluble, lemon-yellow substance, which dissolves in sodium hydroxide solution, with a deep blue colour. The salts of the alkali and alkaline earth metals are readily soluble and acted on by light; solutions of the alkali salts have a blue fluorescence.

Disulphonic Acids of the Aminonaphthols

1-Amino-8-naphthol-2:4-disulphonic acid (SS- or 2S-*acid*), prepared by fusing 1-naphthylamine-2:4:8-trisulphonic acid with sodium hydroxide, is easily soluble in water; ferric chloride colours its aqueous solution a dark green; the *sodium* salt ($1H_2O$) forms small needles; bleaching powder colours its solution brownish-green, becoming reddish-brown with excess of the reagent.

1-Amino-8-naphthol-3:6-disulphonic acid (H-*acid*), prepared by fusing 1-naphthylamine-3:6:8-trisulphonic acid with sodium hydroxide, forms slender needles, sparingly soluble in water; the *diazo*-compound forms sparingly soluble yellow needles which colour a sodium hydroxide solution violet, changing to green on dilution. **Salts:** *sodium hydrogen* ($1\frac{1}{2}H_2O$), slender, white needles, readily soluble in hot water; ferric chloride or bleaching powder solutions give a brownish-red coloration; *barium hydrogen* ($4\frac{1}{2}H_2O$), difficultly soluble silky needles.

Dilute solutions of the acid and salts have a bluish-red fluorescence which on the addition of alkali becomes reddish-violet.

1-Amino-8-naphthol-4:6-disulphonic acid (K-*acid*), prepared by a similar method from the corresponding naphthylaminetrisulphonic acid, unites in acid solution with diazotised sulphanilic acid yielding a very soluble intense Bordeaux-Red azo-dye. The *sodium hydrogen* salt forms needles readily soluble in water, the solution of which has an intense bluish-violet fluorescence, changing to greenish-blue on the addition of alkali; ferric chloride and bleaching powder solutions give dirty green and brownish-red colorations respectively.

2-Amino-8-naphthol-3:6-disulphonic acid (RR- or 2R-*acid*), prepared by fusing 2-naphthylamine-3:6:8-trisulphonic acid with alkalis, is used for making black colours dyeing cotton direct.

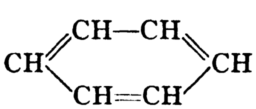
Analysis of the Aminonaphtholsulphonic Acids

The analysis of the aminonaphtholsulphonic acids can be carried out by the method already mentioned for the analysis of naphthylaminesulphonic acids, namely, by titration with standard solutions of sodium nitrite, diazo-benzene or diazo-toluene.

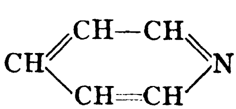
PYRIDINE BASES. C_nH_{2n}–N_6

These bases are contained in coal-tar naphtha; in shale-oil; in peat-tar; in tobacco-smoke; and, together with ammonia and methylamine and its homologues, in the product called "Dippel's oil," obtained by the distillation of bones and other animal matters. Pyridine itself has received several technological applications, and is of great interest theoretically in relation to the alkaloids.

Pyridine may be regarded as benzene, in which one of the CH groups has been replaced by N. Thus:



Benzene



Pyridine

The homologous bases are derived from pyridine by the substitution of CH_3, C_2H_5, etc., for one or more of the hydrogen atoms, and consequently admit of isomeric modification according to the position of the substituted atoms in the chain.

The following is a list of bases of the pyridine series. The b. p. and sp. gr. are only approximate, as the isomeric modifications exhibit sensible differences in their physical properties.

Formula	Base	B. p.	Sp. gr. at 0°	Sp. gr. at 22°
C_5H_5N	Pyridine	115–116°	.9858	....
C_6H_7N	Picoline (2-Methylpyridine)	129–130°	.9613	.933
C_7H_9N	Lutidine	154°	.9443	....
$C_8H_{10}N$	Collidine	179°	.921	....
$C_9H_{13}N$	Parvoline	188°	.906	....
$C_{10}H_{15}N$	Corridine	211°	....	.974
$C_{11}H_{17}N$	Rubidine	230°	....	1.017
$C_{12}H_{19}N$	Viridine	251°	....	1.024

From the above table it is evident that the b. p. rises as the number of carbon-atoms in the molecule increases. For the first four members of the series the sp. gr. diminishes with increase in the molecular weight, but with the higher members the reverse is recorded as being the case. The lower members are miscible with water in all proportions, but collidine and its higher homologues are insoluble, or nearly so, in water.

If a drop or two of pyridine, or one of its homologues, be warmed in a test-tube with a similar quantity of methyl iodide, the product mixed with powdered potassium hydroxide and moistened with water, and heat applied, a highly characteristic and peculiar odour is produced, owing to the formation of a pyridine dihydride. It resembles that of a mixture of mustard oil and isonitrile. The least trace of pyridine or its homologues can be detected in this way. A somewhat similar odour is obtained when a quinoline base is treated in the same manner, but the aniline bases and piperidine do not give the reaction. The foregoing test, due to A. W. Hofmann, is modified by de Coninck as follows: 1 c.c. of the base is gradually mixed with 2 c.c. of methyl iodide, the liquid being cooled during the mixing. The crystalline product is dissolved in about 5 c.c. of alcohol, the liquid

heated to boiling, and very concentrated potassium hydroxide solution dropped in. A blood-red colour is produced, and the liquid finally becomes dark brown if a pyridine base be present (*Compt. Rend.*, 1886, **102**, 1480). Piperidine, sparteine, cicutine, and the aniline bases give no similar reaction.

The bases of the pyridine series are tertiary monamines, and form with alkyl iodides compounds[1] which are not decomposed by potassium hydroxide, but yield alkaline hydroxides by reaction with silver oxide.

The pyridine bases and their salts exert a soporific action on the higher animals. When inhaled, pyridine acts as a respiratory sedative. It has been successfully used as a heat stimulant and as a topical antiseptic in diphtheria. Penzhold found pyridine to act as a general antiseptic, especially as regards *mycelia.* On the lower animals, pyridine and its homologues act as violent poisons, and have been successfully employed in 0.2% solution for destroying the scab-acarus in sheep, the vine-louse, and other injurious insects. The pyridine bases appear to be little, if at all, inferior to nicotine for these purposes, and have also been employed in disinfecting powders.

Isolation of Pyridine Bases

For the *preparation* of the pyridine bases, bone-oil, or the fraction of coal-tar or shale-oil boiling between 80° and 250°, should be agitated with sulphuric acid diluted with twice its measure of water, the treatment being repeated to ensure the complete solution of the bases. The acid liquid is separated and distilled (or boiled by a current of steam) till the vapours no longer redden a slip of fir-wood moistened with hydrochloric acid, showing that all the pyrrole has been driven off. The liquid is then filtered through linen to separate tarry matters, an excess of sodium hydroxide added, and the whole distilled with steam as long as bases continue to pass over, as indicated by the production of fumes on contact of the vapours with hydrochloric acid. The distillate is allowed to cool, and is then treated gradually with a large quantity of solid potassium or sodium hydroxide, till the pyridine bases separate as an oily layer on the surface of the alkaline liquid.[2] The upper stratum is separated, and, if it contains

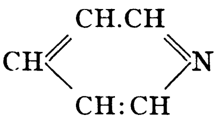
[1] Their methiodides (PyMeI) strongly excite the brain and paralyse the extremities.

[2] The alkali can be greatly economised, with a loss of some of the higher homologues, by rendering the distillate acid with hydrochloric acid, and concentrating it to a small bulk by evaporation at a gentle heat before adding potassium hydroxide.

aniline, fuming nitric acid is cautiously added and the mixture gradually heated to boiling, whereby the aniline is destroyed, while the pyridine bases remain intact.[1] Water is then added, the precipitate filtered off, and the filtrate again treated with solid potassium hydroxide. The layer of bases is removed, and further treated with solid potassium or sodium hydroxide for several days, or until no more alkali dissolves. It is only by prolonged contact with solid caustic alkali that the bases can be freed from water, and it is absolutely necessary to obtain them in a perfectly anhydrous state before attempting to separate them by fractional distillation. This is a very tedious operation, but is greatly facilitated by operating in a vacuum, and by the employment of a Hempel's tube or Henninger's or Glynsky's bulbs. Goldschmidt and Constam (*Ber.*, 1884, **16,** 2976) found that the mixture of bases extracted by vitriol from coal tar boiled between 92° and 200°, and after repeated fractionation a little passed over below 100°, and about one-half between 114° and 117° (pyridine), while above this temperature no constant b. p. was observed. Very little distilled above 160°. The most volatile fraction boiled constantly at 92–93°, treatment of which, with solid potassium hydroxide, caused a separation of absolute pyridine, boiling at 114–115°.

Nearly all pyridine made commercially is now obtained from the so-called "pyridine light oils" which rise to the surface of crude concentrated ammonia liquor made as a by-product from coke oven operations. This oil consists principally of pyridine bases and phenols which are easily separated and the yield of pyridine may amount to 40 to 60% of the total volume of this oil.

Pyridine

$C_5H_5.N$; or 

This substance is the lowest and most important member of the pyridine series of bases. It has been used as an antiseptic and germicide, and is employed in Germany, America and elsewhere for "denaturing" alcohol. Pyridine is the starting-point in the prepara-

[1] Greville Williams destroys aniline and its homologues by heating with potassium nitrite and hydrochloric acid. Häusermann converts the aniline into sulphate, which salt is much less soluble than the sulphates of the other bases.

tion of several valuable antipyretics, and many of the natural alkaloids are derivatives of it.

The method of preparing pyridine from tars has already been sufficiently indicated. It may be obtained by several interesting synthetical reactions, as by passing a mixture of acetylene and hydrocyanic acid through a red-hot tube: $2C_2H_2 + CHN = C_5H_5N$. Pure pyridine is conveniently obtained in small quantity by distilling nicotinic acid with lime: $C_5H_4N.COOH + CaO = C_5H_5N + CaCO_3$.

Commercial pyridine may be purified by dissolving 200 c.c. in 400 c.c. (or a sufficiency) of strong hydrochloric acid, filtering the liquid if necessary, and then adding 1000 c.c. of a 30% aqueous solution of potassium ferrocyanide. The precipitate is filtered off and washed with cold water, in which the hydroferrocyanides of ammonia and the picolines are easily soluble, while the corresponding salt of pyridine dissolves but sparingly. The washed precipitate is treated with a cold, highly concentrated solution of sodium hydroxide, when the pyridine separates as an oily layer; and, thus obtained, it contains a considerable but variable proportion of water, but if desired may be rendered anhydrous by treatment with sticks of potassium or sodium hydroxide. which should be renewed until they cease to liquefy on standing.

The following method of purification has been recommended by Ladenberg (*Annalen*, 1888, **247**, 4): a solution of 135 grm. of mercuric chloride in 1000 c.c. of hot water is added to a solution of 20 grm. of pyridine in 100 grm. of a 10% solution of hydrochloric acid and the precipitated *mercurichloride*, $C_5H_5N,HCl,2HgCl_2$, crystallised from water; it forms compact needles, m. p. 177–178°; the pure double salt is then distilled with a strong solution of sodium hydroxide and the base thus obtained dehydrated in the manner just described.

Pure pyridine is a colourless liquid, b. p. $\frac{115.5^\circ}{760}$ mm., D^{15}_{4} 0.9893, possessing a most powerful and persistent odour, and producing a bitter taste in the mouth and at the back of the throat. The vapour causes severe headache. The b. p. of pyridine is greatly reduced by the presence of water, which it is difficult to separate completely, and which pyridine absorbs with avidity from the air. A mixture of water and pyridine having the composition represented by the formula $C_5H_5N,3H_2O$, has a sp. gr. 1.0219 and boils constantly at 92–93°.

Pyridine dissolves in water in all proportions, but is precipitated from its solutions by excess of strong potassium or sodium hydroxide. It is also miscible with alcohol, ether, chloroform, benzene, and the fatty oils. Pyridine, as compared with its derivatives, is not an active poison; in small doses it has a stimulating effect, while in large doses it has a direct paralysing action on the cardiac muscle.

Pyridine is a powerful base, neutralising acids completely and fuming like ammonia in presence of hydrochloric acid and other volatile acids. It blackens calomel, and precipitates many metallic solutions. Pyridine has no effect on a solution of calcium chloride, but on passing carbon dioxide through the liquid calcium carbonate is precipitated. (No precipitate is produced if aniline be substituted for pyridine in this reaction.) Absolute pyridine has no action on litmus, but in presence of water it turns it strongly blue, though the reaction is not capable of being employed for titrating the base, for which purpose Methyl Orange is suitable. On phenolophthalein pyridine has no action.

Pyridine is an extremely stable substance. It is not affected by treatment with chromic or fuming nitric acid, and these reagents may be employed to free it from aniline and empyreumatic impurities.

A substitution product of pyridine, 3:5-*dibromopyridine*, $C_5H_3NBr_2$, is formed by heating a mixture of pyridine hydrochloride and bromine at 200°. It is precipitated by adding water to its solution in strong hydrochloric acid in needles m. p. 109–110°, but commencing to sublime at 100°. It is soluble in ether and is not acted on by alkalies, acids or oxidising agents.

By reduction with tin and hydrochloric acid, pyridine is converted into piperidine, $C_5H_{11}N$, identical with the substance obtained by hydrolysis of piperine, the alkaloid of pepper.

When pyridine is acted on by sodium at 75–80°, it yields γ-dipyridyl, dipyridine, *iso*-nicotine, etc.

4:4′-(γ)-*Dipyridyl*, $\begin{array}{c} C_5H_4N \\ | \quad\quad | \\ C_5H_4N \end{array}$, crystallises from light petroleum in glistening plates, m. p. 111–112°, b. p. $\frac{293^{\circ}}{744}$ mm., and from water in needles containing $2H_2O$, m. p. 73°, it sublimes in long needles and has a bitter taste. A solution of the *hydrochloride*, $C_{10}H_8N_2$, $2HCl$, which forms large, monoclinic, transparent rods, when treated

with a few drops of a solution of potassium ferrocyanide yields a pale precipitate which quickly becomes dirty indigo-blue and then dissolves in boiling water to a deep purple-red solution.

Dipyridine, $C_{10}H_{10}N_2$, is an almost odourless, highly refractive, viscid oil, b. p. $\frac{286-290^\circ}{735}$ mm. (decomp.), the salts of which are amorphous.

iso-Nicotine, $C_{10}H_{14}N_2$, forms slender needles, m. p. 78°, b. p. above 260° (decomp.); it is very hygroscopic and attacks the skin in the same way as potassium hydroxide. When oxidised with potassium permanganate it yields pyridine-4-carboxylic acid.

Salts of Pyridine

Pyridine forms well-defined salts, most of which are crystallisable and deliquescent. They are odourless when pure, and can be dried without change at 100°, but become slightly coloured on exposure to air and light.

Pyridine nitrate, C_5H_5N,HNO_3, forms slender, colourless needles, or short thick prisms, very easily soluble in water, but less so in alcohol and insoluble in ether.

Pyridine sulphate, $(C_5H_5N)_2,H_2SO_4$, is crystalline, and extremely soluble in water and alcohol.

Pyridine Hydrochloride, C_5H_5N,HCl.—When pyridine is neutralised with hydrochloric acid, and the solution evapourated at 100°, a syrupy liquid is obtained, which, on cooling, becomes gradually converted into a mass of radiating crystals. The salt deliquesces in moist air, and sublimes unchanged at a high temperature. It is volatile to a very notable extent at 100°, and hence cannot be dried at that temperature without loss. It is readily soluble in water and alcohol, but insoluble in ether.

With platinic chloride, a solution of pyridine hydrochloride yields a yellow precipitate of the *platinichloride*, C_5H_5N,H_2PtCl_6, which crystallises in orange-yellow, triclinic prisms, m. p. 240–242°, and decomposes a few degrees above this temperature; it is readily soluble in boiling water, less so in alcohol and insoluble in ether. An aqueous solution of the platinichloride, when boiled for a short time, yields the *salt* $(C_5H_5N)_2,H_2PtCl_6,(C_5H_5N)_2PtCl_2$, crystallising in golden-yellow leaflets, but if the boiling be prolonged for several hours, the *pyridine platinichloride*, $(C_5H_5N)_2PtCl_4$, separates as a yellow pow-

der, insoluble in water and acids. The platinichloride, when heated with an excess of pyridine, yields the *platinosochloride* $(C_5H_5N)_2PtCl_2$, which crystallises from alcohol in small needles.

Pyridine picrate, $C_5H_5N,C_6H_2(NO_2)_3OH$, is deposited in beautiful yellow needles when picric acid in aqueous solution is added to a solution of an equivalent weight of pyridine. The salt has a remarkable tendency to carry picric acid down with it, so that if twice the equivalent proportion of picric acid be employed, the product has the percentage composition of an acid salt, $Py,2\bar{Pc}$; but its real nature is indicated by its behaviour with ether, which dissolves out the free picric acid, leaving the normal picrate. Pyridine picrate may also be prepared by mixing strong solutions of sodium picrate and pyridine hydrochloride. The salt melts at 162°, and is soluble in 91 parts of cold water, but in less than 6 parts of boiling water. It is readily soluble in hot alcohol, but requires about 100 parts of the cold solvent, and is deposited on cooling in long, slender, interlaced needles of a beautiful yellow colour. It is only very slightly soluble in ether, chloroform, or benzene, and practically insoluble in petroleum spirit, but it dissolves with great facility in pyridine and cresylic acid. It is readily soluble, on warming, in ether, benzene, or petroleum spirit containing 10% of cresylic acid, and is freely soluble in aqueous solution of pyridine and sodium cresylate (A. H. Allen).

Pyridine picrate has an intensely bitter taste and nauseous pyridic after-taste. A moderate dose, for example 0.2 grm., produces violent vomiting. It is a valuable insecticide.

Pyridine is remarkable for its tendency to form compounds with metallic salts. These substances are more or less liable to decomposition by washing or boiling with water, and lose pyridine when heated to 100° or a somewhat higher temperature.

The *zinc chloride* compound, $2C_5H_5N,ZnCl_2$, separates as a voluminous white precipitate on treating an aqueous solution of zinc chloride with excess of pyridine; it crystallises from hot alcohol with $2H_2O$ in stout rods, sinters at 200°, and is converted by prolonged treatment with water into pyridine and a basic zinc chloride. The zinc chloride compound dissolves in hydrochloric acid to form a double chloride of zinc and pyridine, $2(C_5H_5N.HCl),ZnCl_2$, which forms groups of white lustrous needles soluble in water, almost insoluble in cold alcohol. The *cupric chloride* compound, $2C_5H_5N,CuCl_2$, is precipitated in fine greenish-blue, glistening, silky needles on adding

pyridine to an alcoholic solution of cupric chloride; it is soluble in pyridine, in aqueous solutions of pyridine and in ammonia. With mercuric chloride, a very dilute aqueous solution of pyridine (1–1000) yields a precipitate which dissolves with extreme readiness in warm water, and separates out, as the solution cools, in long white needles. With mercuric iodide pyridine forms a compound, $2C_5H_5N,HgI_2$, which crystallises from alcohol in beautiful white needles, m. p. 97°.

From acid solutions of pyridine, phosphotungstic acid throws down a very difficultly soluble precipitate.

Detection and Estimation of Pyridine

The recognition and estimation of pyridine are to a great extent based on the properties already described. In the free state, the smell and basic character of pyridine amply suffice for its recognition in the absence of other basic substances of powerful odour, and it is readily liberated from its salts by addition of sodium hydroxide, and obtained free from every interfering substance by distilling its aqueous solution. It may also be extracted from its aqueous solution by agitation with ether, provided that the liquid be saturated with sodium hydroxide.

From *aniline*, pyridine is distinguished by not giving any coloured product on adding a solution of bleaching powder, though the liquid acquires a new and peculiar odour. Means for distinguishing between pyridine and piperidine are given on page 643.

The presence of pyridine in aqueous solutions containing more than 1% may be detected according to E. Vongerichten (*Ber.*, 1889, **32**, 2571) as follows: An alcoholic solution of 1-chloro-2:4-dinitrobenzene is added to a portion of the liquid under investigation and the mixture gently warmed and shaken; after cooling, the addition of sodium hydroxide solution produces a reddish-violet coloration if pyridine is present.

The following method for the detection of pyridine in "denatured" alcohol has been described (*Chem. Ind.*, 1900, **23**, 25). Sulphuric acid is added to the sample of spirit, which is then evapourated to dryness, the residue neutralised with sodium hydroxide solution, distilled and the distillate treated with a solution of potassium mercuric iodide; if pyridine is present, a yellow cystalline precipitate is obtained which, when treated with potassium hydroxide, gives the characteris-

tic odour of pyridine. According to W. Lang, the traces of pyridine sometimes contained in commercial *alcohol* may be detected and removed by shaking the spirit with powdered zinc chloride; or, according to W. Kirschmann, by the addition of an acid solution of aluminium sulphate. In the former case, the pyridine is removed in the form of its zinc chloride compound, and in the latter case pyridine alum is formed. (See Vol. 1.)

The traces of pyridine sometimes present in *fusel oil* may be detected by adding picric acid, which occasions a formation of pyridine picrate.

The presence of *ammonia* in pyridine can be recognised (in the absence of fixed alkalies) by the red coloration produced in the aqueous solution by phenolphthalein, on which pure pyridine has no action. If the indicator be used in considerable quantity, and a low temperature employed (as recommended by J. N. Long, *Analyst*, 1891, **15**, 53) the ammonia can be approximately estimated by titrating the aqueous solution with standard acid.

To detect pyridine in "Liquor Ammonii caustici" Kunze-Krause (*Apoth. Zeit.*, 1910, **25**, 87) recommends that 11 or 12 c.c. of the liquor contained in a test-tube should be neutralised gradually, but as quickly as possible, by adding 5 grm. of powdered tartaric or citric acid, the mixture being constantly stirred. Before and after the addition of the final quantity of acid, the hot liquid is thoroughly shaken and immediately smelt. When the smell of ammonia has disappeared, it should be odourless. Very small quantities of pyridine give a recognisable smell.

Wöhlk (*Ber. deut. Pharm. Ges.*, 1912, **22**, 825) detects pyridine in ammonium salts by grinding about 0.5 grm. of the latter in a mortar with 1 grm. of borax. If pyridine be present, it is immediately recognised by its characteristic odour.

For the detection of traces of pyridine in commercial *ammonia*, H. Ost recommends that the sample should be nearly neutralised, when the odour of pyridine may be recognised. By distilling the nearly neutralised liquid, collecting the distillate in hydrochloric acid, evaporating, and extracting the residue with absolute alcohol, a solution is obtained containing but little ammonium chloride. What is present is removed by boiling off the alcohol and adding platinic chloride solution, when, on evaporating the filtrate and adding alcohol, the pyridine platinichloride crystallises in smooth, ramifying,

orange-red prisms, readily soluble in boiling, but very sparingly in cold, water.

Estimation

A gravimetric method for the estimation of pyridine in aqueous solutions has been described by M. Francois (*Compt. Rend.*, 1903, **137**, 324; *J. Pharm. Chim.*, 1903, **18**, 337). The solution, containing not less than 0.1 grm. of pyridine, is treated with 20–30 drops of hydrochloric acid and an excess of auric chloride, in a small beaker; a precipitate is formed and the solution turns deep yellow. The liquid is evaporated to dryness on a water-bath and when all the hydrogen chloride is driven off, the beaker is placed in a desiccator for a short time. The dried residue is treated with pure dry ether, transferred to a filter and washed with ether until the filtrate runs away colourless, and finally transferred to a weighed crucible; any precipitate adhering to the sides of the beaker is dissolved in a little water and the solution added to the weighed crucible; the water is carefully evaporated on a water-bath and the filter is then incinerated and the ash added to the pyridine aurichloride. The substance is ignited and the residual gold weighed; 196.6 parts of gold correspond to 79 parts of pyridine. Results are quoted in the original which show that the method is accurate.

Pyridine chloraurate, according to G. Bertrand and G. Weissweiler (*Compt. rend.* 1913, **157**, 212) $C_5H_5NH.AuCl_2$, forms yellow needles difficulty soluble in water. The chlorplatinate $(C_5H_5NH)_2PtCl_6$ forms orange yellow spikes and prisms melting at 240–242°, slightly soluble in water. If a dilute solution of pyridine and platinum chloride be boiled, the very slightly water soluble salt $(C_5H_5N)_2PtCl_4$ is formed.

In the absence of ammonia or other bases, free pyridine may be estimated by titration with standard acid and Methyl Orange (not litmus). K. E. Schulze (*Ber.*, 1887, **20**, 3391) recommends the following method, based on the use of ferric chloride as an indicator: Normal sulphuric acid is added slowly and with constant agitation to 20 c.c. of an approximately 5% solution of pyridine, to which has been added previously 1 c.c. of a 5% aqueous solution of ferric chloride, till the precipitated ferric hydroxide is redissolved; toward the end of the action it is advisable to add the acid at the rate of 1 drop per minute. 1 c.c. of normal acid corresponds to 0.079 grm. of pyridine.

A volumetric method based on the absorption of bromine has been devised by A. Labat (*Zeitsch. anal. Chem.*, 1907, **46**, 60). The conditions described must be strictly observed, since the quantity of bromine absorbed depends on the volume of the solution. N/20 solution of bromine in water is added from a burette to 10 c.c. of the solution of pyridine (containing 0.1–5% of pyridine) until an opalescence is produced which persists during 10 secs.; if n = no. of c.c. of bromine-water, then 100 c.c. of the solution contains $\frac{10n}{36} - 0.50$ grm. of pyridine. If more solution be employed for the titration, then for

$$20 \text{ c.c. } x = \frac{10n}{80} - 0.40 \qquad 30 \text{ c.c. } x = \frac{10n}{116} - 0.39$$

$$40 \text{ c.c. } x = \frac{10n}{150} - 0.44 \qquad 50 \text{ c.c. } x = \frac{10n}{200} - 0.415$$

Several methods for the estimation of pyridine in aqueous ammonia have been described. One, devised by Pennock and Morton (*J. Amer. Chem. Soc.*, 1902, **24**, 385) is performed as follows: 100 c.c. of the sample are neutralised with sulphuric acid (1:5), Methyl Orange being used as indicator, with care to keep the temperature below 20°. The liquid is then distilled into a flask containing 30 c.c. of water until the total volume equals 100 c.c.; the distillate, which contains all the pyridine and a small quantity of ammonia, is cooled to 10°, phenolphthalein added and then a solution of mercuric chloride from a burette until the colour is discharged; 4 more drops of the mercuric chloride solution are run in, whereby all the ammonia is carried down in the precipitate of NH_2HgCl; the latter is filtered off and the filtrate titrated with N/10 acid and Methyl Orange; 1 c.c. ≡ 0.0079 grm. of pyridine.

In the analysis of mixtures of ammonia and pyridine, Delépine and Sornet (*Bull. Soc. Chim.*, 1911, [iv] **9**, 706) remove the ammonia by Gerresheim's method, (*Annalen*, 1879, **195**, 373) by precipitating it from solution in hydrochloric acid with mercuric chloride in the presence of sodium carbonate and sodium hydroxide. The pyridine is distilled out of the filtrate, the distillate treated with aurichloride or platinichloride, and the pyridine weighed as the double salt.

Another method, recommended by Milbauer and Stanck (*Zeitsch. anal. Chem.*, 1904, **43**, 215) is as follows: 100–200 c.c. of the

sample are diluted with an equal volume of water and then added to dilute sulphuric acid containing a few drops of a solution of *Patent Blue* V. N. as indicator. The strongly acid liquid is evaporated nearly to dryness and mechanically shaken with a sufficient quantity of freshly prepared sodium hydrogen carbonate solution and an equal volume of ether for 10–15 minutes. The ethereal extract is withdrawn and the aqueous portion again treated with ether. The united ethereal extracts are filtered and thoroughly shaken with an excess of N/10 H_2SO_4 after the addition of a few drops of *Patent Blue*. Sodium chloride is then added in excess and the liquid titrated back with N/10 NaOH solution. Under these conditions the end-point with the indicator is quite sharp. It is advisable to make a third extraction with ether and to titrate the extract as described above to ensure that no pyridine remains in the aqueous layer.

Pyridine bases in ammonium salts are estimated by treating a neutral solution of about 100 grm. of the salt in 30 c.c. of water with sodium hydrogen carbonate and ether, as in the case of the ammonia solutions. If only very slight traces of pyridine are present, a larger quantity of the salt is extracted with hot alcohol, the alcoholic extract acidified and distilled, and the residue treated as just described.

Results of experiments are recorded which show that the method is reliable.

Bayer (*J. Gasbel.*, 1912, **55**, 513) states that in titrating pyridine in ammonium salts, ferric thiocyanate is a more satisfactory indicator than either Methyl Orange or ferric chloride. The mixed bases are acidified with N/10 hydrochloric acid, treated with a drop of ferric chloride solution, and one of ammonium thiocyanate solution, and titrated with N/10 sodium hydroxide solution until the brownish-red colour is destroyed. The bases can be partially separated by distilling the neutral solution of the mixed salts, when the pyridine passes over together with a very small amount of ammonia. The latter is titrated with N/10 hydrochloric acid in the presence of litmus, then excess of acid is added, and the pyridine estimated as above.

A method of estimating pyridine in ammonia water, which depends on the destruction of the ammonia with sodium hypobromite has been described by Houghton (*J. Ind. Eng. Chem.*, 1909, **1**, 698). 100 c.c. of the ammonia water are diluted with 150 c.c. of distilled water in a litre flask and a few drops of Methyl Orange (some

operators prefer freshly prepared cochineal as indicator) solution added. The flask is cooled in running water, and the liquid neutralised with dilute sulphuric acid (1:3) and made slightly acid, then 5 c.c. of N/1 NaOH solution are added, and the liquid distilled (if the proportion of pyridine present is high, either a smaller volume of ammonia water must be used or more than 5 c.c. of N/1 alkali solution added). To destroy the ammonia, the distillate is treated with 100 c.c. of a sodium hypobromite solution (prepared by dissolving 100 grm. of sodium hydroxide in a litre of water and adding 25 grm. of bromine) and shaken until no more gas is evolved. The unchanged pyridine is then distilled into excess of N/10 acid, the excess being titrated with N/10 alkali using Methyl Orange as indicator (1 c.c. N/10 acid is equivalent to 0.0079 grm. pyridine). A more rapid modification of the same method is described by Baessler (*J. Gasbel.*, 1912, **55**, 905) in which the vapour from the slightly alkaline solution of ammonium and pyridine sulphates is made to pass through a sodium hypobromite solution, the vapour from which (containing pyridine only) is collected in the N/10 acid.

A method[1] used in commercial valuation of ammonia liquor in the United States based on the above principles is as follows: Pipette 50 c.c. of ammonia liquor into a Kjeldahl flask, calculating the weight of the sample by multiplying the number of c.c. taken by the sp. gr. of the sample. This may be ascertained by weighing a duplicate 20 c.c. portion, or knowing the ammonia content of the liquor, by referring to a sp. gr. table. The sp. gr. of liquors containing 25–26% NH_3 is approximately 0.910. Add approximately 75 c.c. of distilled water to the flask, and 3 drops of Methyl Orange indicator. Carefully neutralise the ammonia with dilute sulphuric acid (approximately 30%) until just acid, the flask being kept cool under the tap to prevent the escape of pyridine.

Bring the solution just to the point of neutrality with 10% NaOH solution, and add an excess of about 15 c.c. of this caustic solution. Put 300 c.c. of sodium hypobromite solution, (prepared by dissolving 100 grm. of sodium hydroxide in 800 c.c. water, adding 25 c.c. of liquid bromine, shaking until the bromine is entirely dissolved and making up to 1000 c.c.) into a second flask so arranged that on connecting with the Kjeldahl flask the vapours from the Kjeldahl flask will pass through the hypobromite solution, thence through a

[1] Amer. Editors.

condenser and may be collected in a receiver into which latter approximately 25 c.c. of N/10 sulphuric acid solution is placed. Make all connections of the apparatus tight and heat the Kjeldahl flask gently until the Methyl Orange indicates that the solution is becoming acid. This should require from 1¼ to 1½ hours. At no time allow the hypobromite solution to become warmer than 60°. Then boil the solution rapidly until about 200 c.c. of distillate have collected in the receiver, which should require about 45 minutes. Titrate the distillate, using Methyl Orange indicator. When the endpoint is reached add 1 c.c. of phenolphthalein indicator and continue the titration until the first pink tinge lasts for at least 30 seconds. The Methyl Orange titration does not enter into the calculation, but the phenolphthalein titration is a measure of the pyridine present. Run blank test with reagents and apply corrections.

c.c. NaOH (phenolphthalein titration) – c.c. NaOH (blank) times normality, times 7.91, divided by wt. of sample = % pyridine.

It is stated by Fincke (*Zeitsch. Nahr. Genussm.*, 1911, **21**, 655) that a proportion of the spirit used in the manufacture of vinegar has been denatured with pyridine, which accordingly occurs in the resulting product. For its estimation therein Fincke holds that Lunge's method (*Chem. Techn. Unters. Methoden*, 5th Ed., Berlin, 1905, **3**, 583) is inaccurate, and recommends that described by Houghton (*loc. cit.*).

Commercial Pyridine.—Pyridine is employed in Germany,[1] in conjunction with wood spirit and turpentine, for "denaturing" alcohol. An article intended to be used for this purpose is required to answer to the following official tests.

1. The colour must not be deeper than straw-yellow. 2. If 10 c.c. of a solution of 1 c.c. of the sample in 100 c.c. of water be shaken vigorously with 5 c.c. of a 5% solution of anhydrous, fused cadmium chloride, a distinct crystalline deposit should appear within 10 minutes. 3. 10 c.c. of the same solution of pyridine should give a white precipitate with 5 c.c. of Nessler's reagent. 4. When 100 c.c. of the sample are distilled (in a small metal flask provided at the top with a small globe, which is connected with a Liebig's condenser, a thermometer being fitted to the globe, and a moderate heat applied) so that the distillate passes over in separate drops, 90% should have distilled over when the thermometer stands at 140° the barometric

[1] Also in the United States and other countries.

pressure being 760 mm. 5. When the sample is mixed with twice its volume of water it must wholly dissolve, and no oily drops must separate even after long standing. 6. Four drops of the sample heated on platinum foil over a Bunsen flame should burn with a sooty flame and leave no residue. 7. When 20 c.c. of the sample are shaken with an equal volume of a solution of sodium hydroxide of sp. gr. 1.4, a layer of anhydrous base, measuring at least 18.5 c.c., should separate out on standing.

The last test is now usually replaced by one prescribing the use of solid potassium hydroxide. 50 c.c. of the sample are placed in a graduated cylinder, furnished with a stopper, and a long stick of potassium hydroxide immersed in it. The alkali gradually absorbs the water from the pyridine, and forms a lower layer of saturated solution. A second stick is added as soon as the first has sunk much below the surface of the pyridine, and is followed by a third if the second liquefies completely or considerably. Agitation should be avoided, and care must be taken that the last stick is left in contact with the upper layer of bases until the action is at an end. It is then cautiously removed with a bent wire, or broken down by a glass rod, and the volume of the layer of anhydrous bases carefully observed. By this test, commercial pyridine usually shows from 8 to 10% of water (=92 to 90% of anhydrous bases).

Instead of estimating the water, K. E. Schulze recommends titration of the bases with standard acid (*loc. cit.*).

The following test is also employed: N-sulphuric acid solution is added to a solution of 1 c.c. of the sample in 10 c.c. of water until a drop placed on Congo Red paper produces a distinct blue boundary which disappears almost immediately. Not less than 10 c.c. of the acid solution should be used to bring about this result. To prepare the Congo Red paper, filter paper is saturated with a solution of 1 grm. of Congo Red in 1000 c.c. of water and then dried.

Commercial pyridine, as now produced, consists chiefly of pyridine and picoline. Ammonia is apt to be present in notable quantity, as also pyrrole and other strong-smelling impurities.[1] A considerable but variable proportion of water is present.

Commercial pyridine is, as a general rule, by no means pure, boiling over a very wide range and containing, in addition to true pyridine,

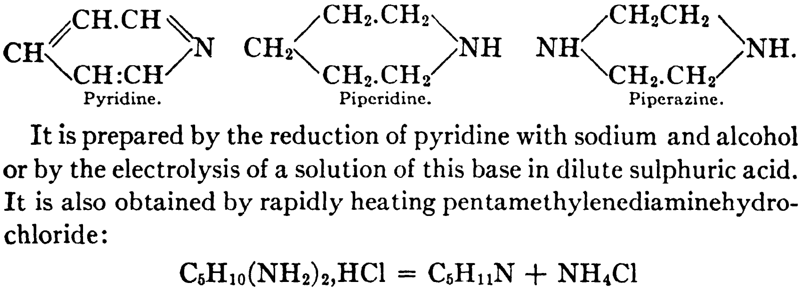
[1] The pyridine produced at certain works becomes turbid when diluted with more than 40% of water, whereas the best makes are miscible with water in all proportions. On distilling the former brands the disturbing impurity is left in the "tailings."

the methyl pyridines, lutidine and some quinoline bases. It is largely used as a denaturant for alcohol and must meet with the approval of the government laboratories. U. S. Government specifications often change, and the reader is referred to the most recent bulletins issued on the subject for exact prevailing specifications.

Pyridine intended for pharmaceutical or medicinal use should not be altered by light; a 10% solution in water should not be reddened by phenolphthalein (presence of ammonia); and 5 c.c., to which 2 drops of N/10 permanganate have been added, should retain a red colour for at least an hour.

Piperidine. $C_5H_{11}N$

The relationship of pyridine to piperidine and piperazine[1] is shown by the following formulæ:



Pyridine. Piperidine. Piperazine.

It is prepared by the reduction of pyridine with sodium and alcohol or by the electrolysis of a solution of this base in dilute sulphuric acid. It is also obtained by rapidly heating pentamethylenediaminehydrochloride:

$$C_5H_{10}(NH_2)_2,HCl = C_5H_{11}N + NH_4Cl$$

Piperidine is likewise produced by the hydrolysis of piperine, the alkaloid of pepper, which when boiled with alkalies, yields piperic acid and piperidine, $C_{17}H_{19}NO_3 + H_2O = C_5H_{11}N + C_{12}H_{10}O_4$.

According to W. Johnstone (*Analyst*, 1890, **14**, 41) a small quantity of piperidine is obtained when pepper is distilled with water alone, probably owing to partial decomposition of the piperine by water or an enzyme.

Piperidine is a colourless, limpid liquid, b. p. 106°; $D^{19.6}$, 0.8619, having a peculiar odour, resembling at the same time that of ammonia and pepper, and possessing a very caustic taste. It dissolves in all proportions of alcohol and water, the addition of water to piperidine being accompanied by the evolution of heat.

[1] *Piperazine* is a strong base which absorbs carbon dioxide from the air and forms large, rhombic leaflets, m. p. 104°, b. p. 145–146°. Piperazine has neither caustic nor toxic properties, and passes through the system unchanged, but dissolves uric acid in large amount, forming the neutral urate, $C_4H_{10}N_2,C_5H_4N_4O_3$. *Piperazine phosphate* forms four-sided tabular crystals, which character, and those of the bismutho-iodide, distinguish piperazine from spermine, $C_4H_8N_2$, which otherwise it closely resembles.

Piperidine is a powerful base. Its aqueous solution restores the blue colour of reddened litmus paper, and behaves like ammonia with metallic solutions, except that the precipitates produced with salts of zinc and copper are not soluble in excess. Piperidine absorbs carbon dioxide from the air, and if the gas be passed into a solution of calcium chloride, to which piperidine has been added, calcium carbonate is precipitated.

Piperidine forms readily crystallisable salts, most of which are soluble; the *hydrochloride*, $C_5H_{11}N,HCl$, forms needles m. p. 237°; the *aurichloride*, $C_5H_{11}N,HAuCl_4$, crystallises from alcohol in four-sided leaflets, sinters at 251°, m. p. 218–219° (decomp.); the *platinichloride*, $C_5H_{11}N,H_2PtCl_6$, forms long, orange needles, m. p. 198–200° (decomp.); it crystallises from alcohol with 1Et.OH in small, orange-yellow needles, m. p. 191° (decomp.).

Piperidine may be distinguished from pyridine by means of the following tests:

Reagent	Piperidine	Pyridine
Freshly prepared solution of gallic acid.	Pale rose coloration turning to deep yellow.	Neither coloration nor precipitate.
Pyrogallol	Yellow coloration at once, gradually changing to brownish-black.	Pale yellow coloration after some time.
Catechol................	Violet coloration changing to pink and finally to yellow.	No coloration.
Quinol..................	Yellow coloration changing to deep brown.	No coloration.

Piperidine is estimated by titration with standard acid, either litmus or Methyl Orange being used as an indicator. 1 c.c. N/10 acid ≡ 0.0085 grm. piperidine.

Homologues of Pyridine

The homologues of pyridine occur with that base in the products of the distillation of bones, coal, shale, etc. Various members of the class have been obtained synthetically.

Picolines. Methylpyridines. C_6H_7N; or $C_5H_4(CH_3)N$

Three isomeric modifications of picoline exist, differing according to the orientation of the CH_3 group in relation to the N. The picoline of coal tar is chiefly the ortho-modification (1:2), often called α-picoline, mixed with some meta- or β-picoline (1:3). Although the former boils at 129° and the latter at 143°, they cannot be separated by fractional distillation, but may be isolated by taking advantage of the different solubilities of their platinichlorides (*Ber.*, 1879, **12**, 2008). Lange (*Ber.*, 1885, **18**, 3436) maintains that α-picoline is preferably separated from bone-oil by means of its sparingly soluble mercurochloride. γ-Picoline (1:4) is produced by the distillation of acroleïn-ammonia, or by heating allyl tribromide with ammonia, and by the action of pyridine on methyl iodide. Its presence has been recognised in coal tar.

The picolines are metameric with aniline, $C_6H_5.NH_2$, which, however, is a primary amine, whereas the picolines have the characters of tertiary bases. In their odour, solubility, basic properties, and characters of their salts, the picolines closely resemble their lower homologue pyridine, but have a lower density and higher b. p. than the latter substance.

α-Picoline, 2-methylpyridine, is an oil, b. p. $\frac{129^\circ}{760}$ mm.; D_4^{15} 0.9497; the *aurichloride* forms small prisms, m. p. about 183–184°; the *platinichloride* forms orange-red crystals, m. p. 194° (decomp.); the *picrate* crystallises in needles, m. p. 169–171°.

β-Picoline, 3-methylpyridine, is an oil, b. p. 143°; D_4^{15}, 0.9613; the *aurichloride* forms yellow needles, m. p. 182–184°; the *picrate* crystallises from alcohol in glistening needles or leaflets, m. p. 149–150°.

γ-Picoline, 4-methylpyridine, is an oil, b. p. 143°; D_4^{15}, 0.9571; the *aurichloride* crystallises in lemon-yellow crystals, sinters at about 185°, m. p. 201–203° (decomp.), the *picrate* forms glistening needles, sinters at 155°, m. p. 163°.

Dimethylpyridines. C_7H_9N

The following dimethylpyridines have been isolated from coal-tar oil, shale oil, etc.

2:4-Dimethylpyridine is an oil, b. p. 159–159.5°; D^{14} 0.9380, soluble in 5 parts of cold water, less soluble in hot water; it is not turned red

by hydrochloric acid or benzoyl chloride; the *platinichloride*, $(C_7H_9N,HCl)_2PtCL_4$, forms orange-red prisms, m. p. 216° when slowly heated, 223° (decomp.) when heated quickly; the *aurichloride*, $C_7H_9N,HAuCl_4$ has m. p. 94°; the *picrate* forms slender, pale yellow needles, m. p. 179°.

3:4-Dimethylpyridine has b. p. 163.5–164.5°: the *platinichloride* ($2H_2O$) forms glistening crystals, m. p. 205° (decomp.); the *aurichloride*, $C_7H_9N,HCl,2AuCl_3$, forms slender, pale yellow needles, m. p. 160–162°.

2:6-Dimethylpyridine has b. p. 142.5°; the *platinichloride* forms orange-red crystals, m. p. 210° (decomp.); the *aurichloride* crystallises in pale yellow, matted needles, m. p. 79–81°; when dried at 80°, m. p. 122–123°; the *picrate* forms slender, pale yellow needles, m. p. 161°.

2:5-Dimethylpyridine has b. p. 154–155°; it is readily soluble in cold, less in warm water; the *platinichloride* ($2H_2O$) forms orange red crystals, m. p. 192–194° (decomp.); the anhydrous salt has m. p. 238° (decomp.); the *picrate* crystallises in yellow needles, m. p. 156–157°.

3:5-Dimethylpyridine has b. p. 169–170°; the *platinichloride* crystallises in dark red needles or leaflets, m. p. 255–256° (decomp.); the *aurichloride* forms yellow needles, m. p. 149°.

Ethylpyridines. C_7H_9N

2-Ethylpyridine occurs in coal-tar oil, it has b. p. 148.5° (corr.); the *platinichloride* forms orange-yellow plates, m. p. 165–167° (decomp.); the *aurichloride* forms glistening, yellow leaflets, m. p. 121°.

3-Ethylpyridine is obtained, together with the 4-isomeride, by heating brucine or cinchonine with potassium hydroxide; it has b. p. 162–165° and is soluble with difficulty in cold water; the *platinichloride* crystallises in glistening, yellowish-red plates, m. p. 196°; the *aurichloride* forms dark yellow leaflets, m. p. 130°; the *picrate* forms yellow needles, m. p. 128–130°.

4-Ethylpyridine is an oil with an unpleasant odour, b. p. 164–166°-the *platinichloride* forms plates m. p. 213°; the *aurichloride* crystallises in prisms, m. p. 147–148°; the *picrate* has m. p. 168°.

Collidines. $C_8H_{11}N$

α-Collidine, 2-methyl-4-ethylpyridine, is an oil, b. p. 178°; D^{15} 0.853, the salts of which are amorphous and gummy; the *platini-*

chloride separates as an oil; the addition of a solution of chromic acid gives a red oil.

β-Collidine, 4-methyl-3-ethylpyridine is an extremely poisonous oil, b. p. 195–196°; the *platinichloride* is an orange-red crystalline powder; the *picrate* has m. p. 148–150°.

γ-Collidine, 2:4:6-trimethylpyridine, occurs in Scottish shale oil; it is an oil, b. p. 171°; D^{15} 0.917, which becomes brown when exposed to the air; the *aurichloride* forms needles, m. p. 112°; the *picrate* forms long, silky, yellow needles, m. p. 155–156°; the *platini-chloride* forms orange-red crystals, m. p. 223–224° (decomp.).

Pyridine-carboxylic Acids

Pyridine itself is an extremely stable substance, resisting the strongest oxidising agents; but its homologues yield by oxidation a series of acids in which the alkyl groups are replaced by a corresponding number of carboxyl groups. The pyridine-carboxylic acids derive their chief interest from the light they throw on the relationship of the natural vegetable alkaloids to the pyridine bases. Three isomeric pyridine-monocarboxylic acids, $C_5H_4N.COOH$, are obtainable, exactly corresponding to the three isomeric modifications of picoline (methyl-pyridine). The same acids may also be obtained by the action of heat on the di- or tri-carboxylic acids, just as benzoic acid, $C_6H_5.COOH$, is obtained by the action of heat (and lime) on phthalic acid, $C_6H_4.(COOH)_2$. One of them (nicotinic acid) is also obtained by the oxidation of nicotine.

Pyridine-monocarboxylic acids, $C_5H_4N.COOH$,[1] unite in themselves the basic characters of pyridine with those of an acid. Thus they combine with hydrochloric acid, and the resulting compound forms double salts with mercuric chloride, platinic chloride, etc.; while, on the other hand, they form a series of well defined crystallisable salts. The following table exhibits their more important characters:

[1] The bases from coal tar boiling between 130° and 140° are boiled in an apparatus furnished with a reflux condenser with 10 times their weight of potassium permanganate in 2½ % aqueous solution, until the permanganate is reduced. The manganese oxide is then filtered off, and the clear liquid concentrated to a small bulk. It is then neutralised and treated with copper acetate. The precipitate is separated, decomposed by hydrogen sulphide and the filtrate decolorised by animal charcoal. On further concentration and cooling it deposits colourless needles of picolinic acid. The filtrate from the copper precipitate is further evaporated, acidified with acetic acid, and treated at its b. p. with copper acetate. The resulting bluish-green precipitate is separated, boiled rapidly with water, and decomposed by hydrogen sulphide. On evaporation, the filtrate deposits colourless crusts of *iso*-nicotinic acid.

	Picolinic or pyridine-2-carboxylic acid	Nicotinic or pyridine-3-carboxylic acid	*iso*-Nicotinic or pyridine-4-carboxylic acid
Mode of formation	Oxidation of α-picoline by permanganate.	Oxidation of β-picoline by permanganate, or nicotine by permanganate, chromic acid or nitric acid.	Action of heat on pyridine di- or tri-carboxylic acid. Oxidation of γ-picoline.
Crystalline character	Prismatic needles	Needles	Needles.
M. p.	135°; sublimes in lustrous needles.	232°	Sublimes in small plates without melting; m. p. in sealed tube 305° (299°) (310°) (317°).
Solubility	Easily soluble in cold or hot water and in alcohol. Nearly insoluble in ether, chloroform, benzene, etc.	Sparingly soluble in cold, easily in warm water; sparingly in ether or chloroform.	Sparingly soluble in water; very sparingly in ether and benzene.
Reaction with neutral lead acetate	No change	No change	
Reaction with ammoniacal lead acetate	No change	White crystalline precipitate.	
Reaction with cupric acetate.	Slowly deposits shining laminæ and needles of violet-blue colour, and metallic lustre. Soluble in hot water.	Pale blue-green precipitate, insoluble in a large quantity of water.	Green crystalline precipitate on warming.
Reaction with ferrous sulphate.	Pale reddish-yellow coloration.	No change	No change.
Characters of hydrochloride— $C_6H_5O_2N.HCl$.	Large, lustrous, orthorhombic prisms which become rapidly turbid on exposure to air.	Monoclinic prisms, quite permanent in the air.	Large glistening, monoclinic prisms.
Aurichloride	M. p. 200°	M. p. 207°	M. p. 219°

On heating with lime, the above acids yield pyridine, just as benzoic acid yields benzene under similar conditions. The sodium salts of the α and β acids, when treated in solution with sodium amalgam, give off ammonia, and yield the salt of an unsaturated acid of the fatty series, $C_6H_8O_3$.

Pyridine-dicarboxylic Acids. $C_5H_3N(COOH)_2$.—Of the six possible acids of this formula, all are known. They are produced by the oxidation of homologues of pyridine containing two substituted hydrogen atoms, and also by the oxidation of other substances.

Quinolinic acid, pyridine-2:3-dicarboxylic acid, obtained by the oxidation of coal-tar quinoline with permanganate, crystallises in

glistening, short, monoclinic prisms; it sinters and turns brown at 190–195°, sometimes also melting at this temperature, but becomes again solid and then has m. p. 231°; the *copper* salt, $(C_7H_4O_4N)_2$-Cu,H_2O, forms microscopic, ultramarine-blue needles. The acid gives a reddish-yellow coloration with ferrous sulphate.

Lutidinic acid, pyridine-2:4-dicarboxylic acid, prepared similarly from 2:4-dimethylpyridine, crystallises in leaflets and plates, m. p. 239–240°; it is moderately soluble in cold, very soluble in hot water, insoluble in benzene and ether, and gives an intense blood-red coloration with ferrous sulphate; the *copper* salt, $C_7H_3O_4NCu,3H_2O$, is obtained as an insoluble, pale bluish-green, crystalline precipitate; the precipitate from a boiling solution is anhydrous.

iso-Cinchomeronic acid, pyridine-2:5-dicarboxylic acid, crystallises from hot water with $1H_2O$ in microscopic leaflets and from cold water with $1\frac{1}{2}H_2O$ in crystals, m. p. 236°; it gives a reddish-yellow coloration with ferrous sulphate; the *copper* salt, $C_7H_3O_4N$-Cu,H_2O is precipitated from hot solutions as a pale-blue, crystalline powder.

Dipicolinic acid, pyridine-2:6-dicarboxylic acid, crystallises from cold water with $1\frac{1}{2}H_2O$ in long, slender, silky needles, m. p. 226° (decomp.); it gives a reddish-yellow coloration with ferrous sulphate; the *copper* salt, $C_7H_3O_4NCu,2H_2O$, forms dark blue prisms.

Cinchomeronic acid, pyridine-3:4-dicarboxylic acid, is obtained by the oxidation of quinine, cinchonine, etc., by nitric acid and by the oxidation of *iso*-quinoline with potassium permanganate; it crystallises in granules from water and in prisms from acidified solutions, m. p. 258–259° (decomp.); the *copper* salt, C_7H_3NCu, $3\frac{1}{2}H_2O$, is a dark blue, crystalline precipitate which loses $3H_2O$ at 100°.

Dinicotinic acid, pyridine-3:5-dicarboxylic acid, has m. p. 323° (decomp.); it is almost insoluble in water.

Pyridine-tricarboxylic acids, $C_5H_2N(CO_2H)_3$, are obtained by the oxidation of certain alkaloids. Thus cinchonine, cinchonidine, quinine and quinidine, when oxidised by an alkaline solution of potassium permanganate, yield *α-carbocinchomeronic* or *pyridine-2:3:4-tricarboxylic acid*, which crystallises with $1\frac{1}{2}H_2O$ in transparent, rhombic plates, loses its water of crystallisation at 115–120° and then has m. p. 249–250° (decomp.), when heated rapidly; it yields cinchomeronic acid when heated at 170° and gives a pale-red coloration with ferrous sulphate. Berberine, when oxidised by nitric acid, yields

berberonic or *pyridine-2:4:5-tricarboxylic acid,* which crystallises with $2H_2O$ in triclinic prisms, loses $1H_2O$ when exposed to the air, turns red at 215° and has m. p. 235°; it yields with ferrous sulphate a blood-red coloration and with lead acetate an insoluble precipitate.

Pyrrole. $$\begin{array}{l} \mathrm{CH:CH}\diagdown \\ \vert \qquad\qquad \mathrm{NH.} \\ \mathrm{CH:CH}\diagup \end{array}$$

This associate of the pyridine bases in coal tar and bone oil is widely distributed in nature, since chlorophyll, the green pigment of plants, and hæmoglobin, the pigment of blood, are derivatives of pyrrole. It is best prepared by shaking bone oil with dilute sulphuric acid and fractionating the insoluble portion. The portion, b. p. 98–150°, is heated with potassium hydroxide solution so long as ammonia is evolved and then steam-distilled; the portion of the distillate, b. p. 115–130°, is heated with an excess of solid potassium hydroxide until two layers form; it is then allowed to cool, the oil poured off and the potassium pyrrole, C_4H_4NK, washed with ether and decomposed by water; the liberated pyrrole is separated by steam-distillation and fractionated.

Pyrrole is a colourless liquid, b. p. 130–131°; D_4^{21} 0.96694, with a pungent taste, and odour resembling chloroform. It is but little soluble in water, and insoluble in alkalies, but dissolves in dilute acids alcohol, and ether. It is indifferent to most reagents, but appears to possess feebly-marked basic properties. The only definite salt is the *picrate,* which forms unstable red needles melting at 71°.

Pyrrole turns brown in the air, and when warmed with acid forms a red substance known as pyrrole red. A piece of pine-wood, moistened with hydrochloric acid and exposed to the vapour of pyrrole becomes faintly red, and after some time, carmine-red.

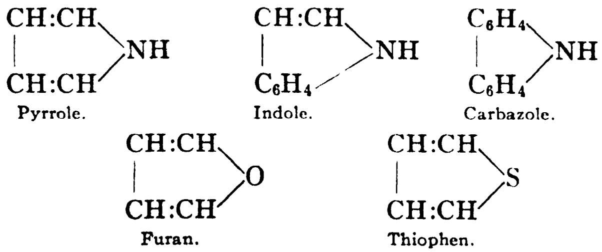
When 5 grm. of pyrrole are added to a solution of 10 grm. of isatin in 500 c.c. of water and 50 grm. of sulphuric acid at 5°, an indigo-blue substance, *pyrrole-blue A,* $C_{24}H_{16}O_3N_4$, is obtained, which dissolves in concentrated sulphuric acid to a violet solution changing to bluish-black. A similar compound, *pyrrole-blue B,* $C_{24}H_{16}O_2N_4$, is obtained by adding a solution of 0.75 grm. of pyrrole in 10 c.c. of glacial acetic acid to a mixture of 100 c.c. of a 1% solution of isatin in glacial acetic acid and 40 c.c. of 15% sulphuric acid cooled to 0°, and diluting the mixture after 5 minutes with 10 c.c. of ice-water; the dried precip-

itate, after boiling 2 or 3 times with pyridine, is obtained as a glistening, blue powder resembling cantharidine; it dissolves in boiling glacial acetic acid to a blue solution and in concentrated sulphuric acid to a violet-red solution which changes to blue and on the addition of water deposits a disulphonic acid.

Small quantities of pyrrole may be detected by boiling a short time with 2 c.c. of a solution of alloxan; if pyrrole is present, a violet-blue coloration is produced which becomes red when the solution is cooled with cold water, and on the addition of aqueous sodium hydroxide turns green changing rapidly to an intense blue.

A 4% solution of formaldehyde containing a few drops of sulphuric acid, when treated with pyrrole in the cold, yields in a few minutes a white substance which decomposes without melting when heated, is insoluble in all organic solvents and turns red when exposed to the air.

If a solution of phenanthraquinone in acetic acid be treated with pyrrole and a little dilute sulphuric acid, a brown precipitate is formed, which dissolves in chloroform with a beautiful violet-red colour. When an aqueous solution of benzoquinone is treated with pyrrole and dilute sulphuric acid, a dark green precipitate is formed, insoluble in ether. These reactions indicate the close relationship between pyrrole and thiophen, which itself has the constitution of a thiofuran. Many of the reactions of pyrrole are also produced by carbazole, which is in imino-diphenyl. Indole has a constitution between pyrrole and carbazole. Thus:



Pyrrole. Indole. Carbazole.

Furan. Thiophen.

Pyrrole

Herzfeld describes (*Biochem. Zeit.*, 1913, **56**, 82) the following simple test for pyrrole, which has the advantage of distinguishing it from indole; an indole solution, when treated with solutions of sodium hydroxide and sodium nitroprusside, acquires a violet blue

colour, which on addition of acetic acid becomes blue. Under the same conditions a pyrrole solution gives a brownish-red colour, which, unlike the violet blue of indole, can be extracted with chloroform. With a solution containing both indole and pyrrole, after shaking with chloroform, a liquid is obtained which consists of a blue upper layer and a brownish-red lower layer.

Methylpyrroles

Two isomeric methylpyrroles exist in bone-oil. To isolate these, the fraction of bone oil, b. p. 140–150° is converted into the potassium derivative and this is heated at 200° in a current of carbon dioxide. Two isomeric *homopyrrolecarboxylic acids* are formed. The α-acid forms leaflets, m. p. 169.5° and a *lead* salt very soluble in water, whilst the β-acid forms crystalline crusts, m. p. 142.4° and a nearly insoluble *lead* salt. On distilling the respective acids with lime the corresponding homopyrroles are regenerated. The α-compound, 2-*methylpyrrole*, is a liquid, b. p. $\frac{147-148^\circ}{750}$ mm., whilst the isomeride, 3-*methylpyrrole*, is a liquid, b. p. $\frac{142-143^\circ}{742.7}$ mm.

2:5-Dimethylpyrrole also occurs in bone oil; it is a liquid, b. p. 169°, with a sharp, unpleasant odour, gives a cherry-red coloration with a pine-shaving moistened with hydrochloric acid, and a brownish-red coloration with ferric chloride.

Tetraiodopyrrole, C_4I_4NH, has been introduced into medicine under the name of "*iodol.*" It is prepared by the action of a solution of iodine in potassium iodide on a solution of pyrrole containing potassium hydroxide, and forms a tasteless, pale yellow, crystalline powder, having a faint, thymol-like odour. Iodol decomposes without melting at 140–150°, is soluble in 5000 parts of water, moderately soluble in light petroleum and dilute acids, readily soluble in ether and hot alcohol. Iodol is not decomposed by boiling water, but is turned black by hydrochloric acid; an alcoholic solution does not give a precipitate with mercuric chloride.

An *additive* compound, $C_4I_4NH,C_{10}H_{18}O$, is formed by warming cineol (eucalyptol) with iodol; it forms yellowish-green crystals, m. p. 112° (decomp.).

Iodol contains 90% of iodine and possesses antiseptic and local anaesthetic properties analogous to those of iodoform, over which its

slight odour and freedom from toxic properties give it the preference. Iodol can be recognised by the green colour of its solution in sulphuric acid, changing to dirty violet and by the bright red colour produced when an alcoholic solution is warmed with nitric acid.

A compound of iodol with egg albumin has been introduced recently into medicine for internal administration; it is an odourless, tasteless, pale yellow powder, insoluble in the ordinary solvents, and soluble only in dilute alkalies.

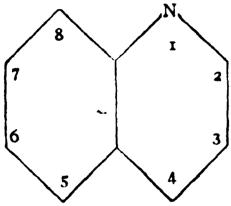
Quinoline and Its Allies

The interesting base which gives its name to the quinoline series bears the same relation to naphthalene that pyridine bears to benzene; that is, it is derived by the substitution of an atom of nitrogen for one of the CH groups of naphthalene:

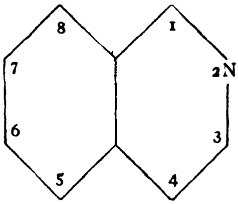
Benzene,	C_6H_6	Pyridine,	C_5H_5N
Naphthalene,	$C_{10}H_8$	Quinoline,	C_9H_7N

Just as two isomeric naphthols exist, so two isomeric quinolines are possible and have been obtained. Quinoline (I) is obtained with its homologues by distilling quinine, cinchonine and other alkaloids with lime or potassium hydroxide; it also exists, together with *iso*-quinoline (II), in coal-tar.

I.



II.



Quinoline, Chinoline, Leucoline. C_9H_7N

This base is generally prepared by Skraup's method, which consists in shaking together aniline (76 pts.), glycerol (240 pts.), sulphuric acid (200 pts.), and nitrobenzene (48 pts.) or arsenic acid (114 pts.). When the aniline sulphate has dissolved, a reflux condenser is fitted to the flask, which is heated at 130° until action commences when the flame is removed. When the action is at an end (3 hours), the product is cautiously diluted with water, boiled to get rid of traces of nitrobenzene, then rendered alkaline, and the quinoline and aniline

distilled in a current of steam. The oil obtained is separated from the aqueous layer, dehydrated over potassium hydroxide, and fractionally distilled, whereby a separation of the bases is effected tolerably readily, aniline boiling at 184°, and quinoline at 239°. To purify the latter it is again fractionally distilled, and boiled with weak chromic acid mixture (to oxidise any aniline); or the quinoline is dissolved in six parts of water, and strong sulphuric acid added in the exact quantity necessary to combine with the base. After cooling, the liquid is filtered, and the insoluble hydrogen sulphate washed with alcohol till snow-white, and then decomposed by potassium hydroxide.

If arsenic acid be used instead of nitrobenzene, after the steam distillation the distillate is treated with excess of hydrochloric acid and then with sodium nitrite, warmed, saturated with sodium hydroxide and again steam-distilled. The distillate is then extracted with ether. (Knüppel, D. R. P. 14976.)

Quinoline is a colourless, mobile liquid, m. p.—19.5°, b. p. $\frac{237^\circ}{746}$ mm. (Skraup), $\frac{240-241^\circ}{750}$ mm. (Kretsky), $\frac{238^\circ}{760}$ mm. (Kahlbaum); D_0^0 1.1081, D_{20}^{20} 1.0947, having a penetrating and peculiar taste and an after-taste slightly resembling peppermint oil. It has a faint aromatic odour, like that of bitter-almond oil. Quinoline evaporates completely, but slowly, at the ordinary temperature, so that the grease spot formed by it on paper is not permanent. It resinifies on exposure to air, and when left standing over water forms a mixture having the composition $C_9H_7N,1\frac{1}{2}H_2O$, which becomes turbid at blood temperature. Quinoline containing traces of water boils at 227–228°.

Quinoline is very sparingly soluble in cold water, but more freely so in hot. It is miscible in all proportions with alcohol, ether, carbon disulphide, and fixed and volatile oils; and is also easily soluble in chloroform, amyl alcohol, benzene and light petroleum.

Quinoline has well-marked basic characters, and forms an extensive series of salts, most of which are crystallisable and deliquescent. It precipitates ferric and aluminium solutions, and at a high temperature decomposes ammonium salts.

Salts: the *hydrochloride*, C_9H_7N,HCl, forms small aggregates of hygroscopic crystals, m. p. 93–94°; the *platinichloride*, C_9H_7N,H_2PtCl_6, crystallises from hot dilute hydrochloric acid with $2H_2O$ in yellow needles, m. p. 225°, and from hot water with $1H_2O$ in small

yellow needles, m. p. 218°; the *picrate* forms bright yellow needles, m. p. 205°; the *tartrate*, $3C_9H_7N,4C_4H_6O_6$, forms large, flat, rhombic needles, m. p. 125° (decomp.), and is readily soluble in water and hot alcohol; it is used as an antipyretic and antiseptic, being specially useful in the case of intermittent fevers; the *salicylate*, C_9H_7N,C_7HO_3, is a greyish-white powder soluble in water, alcohol and ether; it is employed as an antiseptic and antineuralgic; the *thiocyanate*, $C_9H_7N,HCNS,xH_2O$, is used for certain venereal diseases, but finds greater application in the form of the bismuth double salt, $(C_9H_7N,\text{-}HCNS)_2,Bi(SCN)_3$, under the name of "*crurin*," a reddish-yellow powder insoluble in alcohol, ether and water; it is usually taken in the form of tablets containing 50% of starch.

Quinoline forms additive compounds with many organic substances. Thus, an ethereal solution of the base and iodoform, when kept a few hours, deposits the *additive product*, $3C_9H_7N,CHI_3$, in the form of large needles, m. p. 65°, insoluble in water, acids and alkalies, soluble in benzene and light petroleum; it is used as an antiseptic and antipyretic. The *additive product* with *iso*-amyl iodide, $C_9H_7N,C_5H_{11}I$, forms yellowish-green crystals, m. p. 184–185°; if the quinoline employed contains lepidine (as is the case with quinoline made from cinchonine) the additive product, when dissolved in aqueous potassium hydroxide, gives a beautiful, but not very permanent, blue colour owing to the formation of *Quinoline-blue*, $C_{28}H_{35}N_2I,1\frac{1}{2}H_2O$. The latter substance crystallises in green needles with a metallic reflex, m. p. 100°, when heated quickly, and dissolves in alcohol to a beautiful blue solution. Both quinaldine and lepidine give this reaction.

Quinoline (2 mols.) heated with resorcinol (1 mol.) at 100° yields a *substance* which crystallises in silvery plates, m. p. 102°, and is readily soluble in alcohol, ether and chloroform, but insoluble in light petroleum; it is used as an antiseptic and antipyretic.

Reaction of Quinoline and Its Salts

Quinoline salts in aqueous solution are precipitated milky white by alkali hydroxides and ammonia, the precipitate being somewhat soluble in excess. From the alkaline liquid, the quinoline can be readily extracted by ether, chloroform, or petroleum spirit.

Iodised iodide of potassium gives a reddish-brown precipitate even in dilute solutions of quinoline salts (1 in 25,000). Potassio-mercuric

iodide only precipitates quinoline from tolerably strong solutions (1 in 3000), the precipitate being yellowish-white and amorphous, but converted into delicate amber-yellow needles on addition of hydrochloric acid. This reaction is characteristic. Phosphomolybdic acid, in presence of nitric acid, produces a yellowish-white precipitate in quinoline solutions.

Potassium ferrocyanide colours solutions of quinoline salts greenish, and on addition of hydrochloric acid a reddish-yellow amorphous precipitate is thrown down, if the liquid be not too dilute.

Quinoline is precipitated by picric acid, but not by tannic acid or ferric chloride; and its salts, in the solid state, yield no colour reactions with nitric acid or strong sulphuric acid, either alone or in association with oxidising agents.

A solution of a quinoline salt, when treated with a solution of potassium dichromate, yields a yellow, crystalline precipitate of the *dichromate*, $C_9H_7N,H_2Cr_2O_7$, which crystallises from water in glistening needles, m. p. 164–167°.

Quinoline possesses powerful antiseptic properties; 0.2% of the tartrate is said to prevent the lactic fermentation of milk completely, the decomposition of urine and gelatin, and the development of bacteria in cultivation fluid. Even in concentrated solution it does not coagulate albumin, and in the proportion of 1% it completely destroys the coagulability of the blood. On the other hand, quinoline is remarkably inactive to yeast-cells, and does not affect alcoholic fermentation, even when present in considerable quantity.

Quinoline has been used in medicine as an antipyretic, the adult dose of the tartrate being from 7 to 12 gr. It is said by some not to produce any unpleasant after-effects, but by others to cause irritation of the stomach and collapse. It is not found in the urine of those who have taken it internally.

An aqueous solution of quinoline is used as a gargle for diphtheria and dysentery.

Pure quinoline should be colourless or only faintly yellow, and have the correct b. p. If mixed with 40–50 times its own weight of water, it should give a filtrate which is not coloured violet by a solution of bleaching powder.

The salts of quinoline should be completely soluble in water, and the free base in a slight excess of hydrochloric acid. The neutral solution should be free from bitter taste (which indicates the presence

of impurity derived from cinchonine), and should not give a coloured precipitate with alkali hydroxides.

Estimation of Quinoline

Quinoline can be titrated fairly accurately with standard acid, if Methyl-Orange be employed as an indicator.

Tetrahydroquinoline. $$C_6H_4 \left\langle \begin{array}{l} CH_2.CH_2 \\ \quad\quad\quad | \\ NH.CH_2 \end{array} \right.$$

When quinoline is acted on by nascent hydrogen, it is first converted into *dihydroquinoline*, C_9H_9N, a solid substance, m. p. 161°, and subsequently into *tetrahydroquinoline*, a liquid, b. p. 251°, the *hydrochloride* of which, $C_9H_{11}N,HCl$, forms prisms, m. p. 180–181°, whilst the *platinichloride* forms reddish-yellow crystals, m. p. 200°. Both these reduction products yield nitrosamines, and can be alkylated, and hence are secondary bases. Tetrahydroquinoline possesses stronger antipyretic characters than quinoline itself, and this property is exhibited still more strongly in certain of its derivatives, several of which have received some application in medicine (see below).

Alkylquinolines

A large number of the higher homologues of quinoline are produced on distilling alkaloids with potassium hydroxide while many have been isolated from coal tar and shale oils.

From the acid tar produced in the purification of shale oil, Robinson and Goodwin (*Trans. Roy. Soc. Edin.*, 1879, **28**, 561; 1880, **29**, 265) obtained the following bases of the quinoline series.

Base	Formula	B. p.
Tetracoline	$C_{12}H_{13}N$	290–295
Pentacoline	$C_{13}H_{15}N$	305–310
Hexacoline	$C_{14}H_{17}N$	325–330
Heptacoline	$C_{15}H_{19}N$	345–350
Octacoline	$C_{16}H_{21}N$	360–365

Quinaldine, 2-methylquinoline, C_9H_6MeN, sometimes forms 25% of coal-tar quinoline; it is a colourless liquid with a faint quinoline-like odour, b. p. $\frac{244-245^\circ}{750}$ mm., the salts of which are mostly soluble in water; the *dichromate*, $(C_{10}H_9N)_2,H_2Cr_2O_7$, forms yellowish-red needles, soluble with difficulty in cold, readily soluble in hot, water. When heated with phthalic anhydride and zinc chloride, quinalidine gives rise to a beautiful yellow dye, *quinophthalone*, $C_6H_4(CO)_2:CH.NC_9H_6$, m. p. 235°. The sodium salt of the sulphonic acid of the latter substance is the *Quinoline Yellow* of commerce.

Quinaldine has been used as an antipyretic and antiseptic, but has a much weaker effect than quinoline.

Lepidine, 4-methylquinoline, is obtained together with quinoline when cinchonine is distilled with potassium hydroxide. It is a liquid with an odour like quinoline, b. p. 261–263°; D^{20} 1.0862, and solidifies to a crystalline mass at 0°. It is readily soluble in water and miscible with alcohol, benzene, ether, and light petroleum in all proportions. It closely resembles quinoline in its antipyretic and antiseptic properties.

8-Hydroxyquinoline, $OH.C_9H_6N$, is of importance, since many antipyretics and antiseptics are derived from it (see below). It is obtained by fusing quinoline-8-sulphonic acid with sodium hydroxide, also by Skraup's reaction from 2-aminophenol, 2-nitrophenol, glycerol and sulphuric acid.

8-Hydroxyquinoline crystallises in long, glistening prisms, having a saffron-like odour, m. p. 75–76°, b. p. $\frac{266.6^\circ \text{ (corr.)}}{752 \text{ mm.}}$; it is volatile in steam, sublimes slowly at the ordinary temperature, is soluble with difficulty in ether and cold water, but readily soluble in alcohol, benzene, chloroform and dilute sodium hydroxide. The solutions in acids and alkalies are yellow; the colourless alcoholic solution becomes yellow on the addition of water. An aqueous solution gives with ferric chloride an intense green coloration and with ferrous sulphate a red coloration followed by a black precipitate.

An alcoholic solution of 8-hydroxyquinoline, when treated with copper acetate, yields a greenish-yellow crystalline precipitate of the *copper* salt, $(C_9H_6ON)_2Cu$. The *picrate*, $C_9H_7ON,C_6H_2(NO_2)_3OH$,

forms yellow prisms, m. p. 203–204°; it is difficultly soluble in cold alcohol and almost insoluble in benzene.

Carbostyril, 2-hydroxyquinoline, $C_9H_6(OH)N$, is used in medicine and in large doses has a similar action to curare. It is obtained by the reduction of *o*-nitrocinnamic acid and crystallises with $1H_2O$ from a hot 1% aqueous solution in long, feathery crystals and from alcohol in large prisms, m. p. 199–200°. It dissolves in aqueous alkalies forming salts which are decomposed by carbon dioxide.

Antipyretics and Antiseptics Derived from Quinoline

A considerable number of substances related to quinoline, and mostly allied to tetrahydroquinoline, have been introduced from time to time as antiseptics, antipyretics and febrifuges. Some of these are very powerful in their action and appear likely to receive a permanent place in medicine; but they are not periodics, and cannot be substituted for quinine in cases of ague or intermittent fevers. The following are some of the most important of the antipyretics and antiseptics derived from or related to quinoline.

M-Kairoline is the hydrogen sulphate of 1-methyltetrahydroquinoline, $C_6H_4\begin{array}{l}\diagup CH_2.CH_2\\ \qquad\qquad |\\ \diagdown NMe.CH_2\end{array}$, obtained by acting on tetrahydroquinoline with methyl iodide; the free base has b. p. 245°.

A-Kairoline has a similar constitution, but contains an ethyl instead of a methyl group.

M-Kairine is the hydrochloride of 8-hydroxy-1-methyltetrahydroquinoline, $OH.C_6H_3\begin{array}{l}\diagup CH_2.CH_2\\ \qquad\qquad |\\ \diagdown NMe.CH_2\end{array}$. The corresponding ethyl compound is known as A-*Kairine*. On adding an alkali hydroxide to the aqueous solution of a kairine, the penetrating characteristic odour and bitter taste of the free base are easily recognised, while the alkaline solution rapidly becomes coloured and deposits a brown humus-like substance. When the aqueous or alcoholic solution of a kairine is treated with an oxidising agent, such as potassium dichromate and an acid, it gives a series of colours ranging from violet-blue to purple, or sometimes greenish. Without the addition of an acid, the solution becomes dark purple, and on standing a violet precipitate is formed, which dissolves in alcohol with black colour. A drop

of ferric chloride, added to a dilute and neutral solution of kairine, instantly produces a violet coloration, rapidly changing to brown, with precipitation. An excess of ferric chloride added to a strong solution of kairine produces a nearly black precipitate. Sodium nitrite and dilute sulphuric acid produce an orange or red colour in kairine solutions. Potassium ferrocyanide gives a voluminous precipitate, and phosphotungstic acid a pale yellow precipitate.

The kairines act as powerful antipyretics. Their use is almost obsolete, as their action is somewhat uncertain; and they are said to be liable to produce vomiting, cyanosis, and collapse.

Thalline is the commercial name of another antipyretic having the constitution of 6-methoxytetrahydroquinoline, $OMe.C_6H_3\left\langle\begin{array}{l}CH_2.CH_2\\ \quad\quad\;\;\,|\\ NH.CH_2\end{array}\right.$.

Thalline is prepared by heating *p*-aminoanisole and *p*-nitroanisole with glycerol and sulphuric acid and reducing the product with nascent hydrogen. The free base crystallises in large, colourless prisms, m. p. 42°, b. p. 283°, possesses a bitter, saline and pungent taste, and is sparingly soluble in water, but readily soluble in alcohol, benzene, chloroform or ether.

Thalline sulphate, $(C_{10}H_{13}ON)_2,H_2SO_4,2H_2O$, is the most common variety of commercial thalline. It crystallises from alcohol in long, colourless needles having an aromatic, bitter, saline taste and a faint odour resembling coumarin. It dissolves in 7 parts of cold water and 100 parts of 90% alcohol; the solutions give an acid indication and become brown on exposure to light. A very dilute aqueous solution of commercial thalline gives with ferric chloride a yellow coloration, changing to emerald-green (destroyed by reducing agents) and passing in a few hours to deep red. The reaction is extremely delicate. A green colour is also produced by auric chloride, silver nitrate, mercuric nitrate, chlorine water, etc., and, in acid solution, also by a solution of bleaching powder and potassium ferricyanide. Strong sulphuric acid dissolves thalline sulphate without coloration, but on addition of nitric acid the liquid becomes deep red, and immediately afterward yellow-red. Fuming nitric acid colours a dilute aqueous solution red. Sulphuric acid and sugar give a red coloration. Iodine colours the solution dark brown, then dingy green. Ammonia forms a white precipitate of the free base, readily taken up by ether on agitation. If not too dilute, solutions

of thalline sulphate yield precipitates with the general reagents for alkaloids.

If to an aqueous solution of β-naphthaquinone a small quantity of the solution of a thalline salt be added, and then a drop or two of sodium hydroxide solution, a fine cherry-red coloration is produced, becoming more brilliant on adding nitric acid. The colouring matter is extracted by ether or chloroform.

Thalline tartrate occurs in commerce as a faintly yellow crystalline powder. It dissolves in 10 parts of cold water, and the solution gives the same reactions as the sulphate. In alcohol it is very sparingly soluble. The salt contains 52.2% of thalline.

The salts of thalline become altered by exposure to light.

Thalline salts are powerfully antipyretic, and have been employed in yellow fever. They cause profuse perspiration, and are apt to produce depression, etc. Hence their internal use is practically obsolete. Thalline acts as a direct blood-poison, its antithermic properties being due to the destruction of the red corpuscles. It has found considerable application in the treatment of gonorrhœa. The sulphate was official in the *German Pharmacopœia* of 1890.

Exhibition of thalline causes a dark coloration of the urine. A derivative, which also gives a green colour with ferric chloride, but differs from thalline in being extracted by agitating the acidified urine with light petroleum, should first be removed, and then the unaltered portion of the thalline can be isolated by rendering the urine alkaline with ammonia, and agitating with ether or benzene. Very small quantities of thalline can in this way be recognised in urine.

An *additive* product of thalline (or its sulphate) and iodine has been introduced for the treatment of carcinoma.

Thermifugin, another antipyretic, is sodium hydroxy-1-methyl-tetrahydroquinolinecarboxylate,

$$CO_2Na.C_6H_2(OH)\left\langle\begin{array}{l} CH_2.CH_2 \\ \quad\quad\;\; | \\ NMe.CH_2 \end{array}\right. .$$

It forms colourless crystals which dissolve readily in water; the solutions become brown when kept.

Analgen, 5-acetylamino-8-ethoxyquinoline,

$$\begin{array}{l} CH.:C(NHAc).C.CH:CH \\ | \qquad\qquad\qquad\;\; \| \qquad | \\ CH:C(OEt).-C-N:CH, \end{array}$$

forms colourless crystals, m. p. 155°; it is readily soluble in alcohol, sparingly soluble in water (7 in 1000); an acidified aqueous solution is yellowish-red.

The corresponding benzoyl compound, $NHBz.C_9H_5(OEt)N$, is known as *benzanalgen*, *quinalgen* and *labordin;* it is a white, tasteless, crystalline powder, m. p. 208°, readily soluble in very dilute acids; the solutions are coloured.

The analgens find application in the treatment of lumbago, rheumatism and neuralgia.

Loretin is the name given to 7-iodo-8-hydroxyquinoline-5-sulphonic acid, $\left.\begin{array}{l} CH:C(SO_3H) \\ | \\ CI = C(OH) \end{array}\right\rangle C_5H_3N$,
an odourless, tasteless, reddish-yellow, crystalline powder, which turns brown at 200° and liberates iodine at 260°. It is readily soluble in alcohol, ether and water and dissolves without decomposing in hot concentrated sulphuric acid. The sodium salt is compressed into tablets, which dissolve in 11 parts of hot water to a yellow solution, and is used in this form for making disinfecting baths.

A 5–10% solution of loretin in collodion is employed for coating wounds.

A non-poisonous compound of loretin with iodoform has been introduced for the treatment of wounds.

A mixture of the sodium salt of loretin (10 pts.) with bismuth nitrate (4 pts.), occurring as an insoluble, yellow powder, finds application as an antiseptic and astringent.

Griserin is a mixture of 20% of sodium hydrogen carbonate and an iodohydroxyquinolinesulphonic acid; it dissolves in water, but is insoluble in alcohol, ether and chloroform. It is employed as an antiseptic in the treatment of tuberculosis and other infectious diseases.

Quinosol is the double sulphate of potassium and 8-hydroxyquinoline, $(OH.C_9H_6N)_2,H_2SO_4,K_2SO_4$. It is a crystalline, sulphur-yellow powder which dissolves readily in water. Ferric chloride gives an intense dark-green coloration. Quinosol is employed in gynecological operations.

Argentol is the silver salt of a hydroxyquinolinesulphonic acid, $OH.C_9H_5N.SO_3Ag$. It is employed in the form of an ointment with

vaseline or lanoline for the treatment of ulcers, wounds and various skin diseases.

Diaptherin is a compound formed by the union of 2 mols. of 8-hydroxyquinoline with 1 mol. of *o*-phenolsulphonic acid, $OH.C_6H_4.SO_3H,2C_9H_6N.OH$. It forms transparent, yellow crystals, m. p. 85°, is soluble with difficulty in absolute alcohol and moderately soluble in water. Ferric chloride produces a bluish-green coloration which becomes yellow on the addition of hydrochloric acid. Diaptherin is non-poisonous; it is used as an antiseptic and for the treatment of rheumatism.

Cinchophen Phenylcinchoninic Acid.—2-phenyl-quinoline-4-carboxylic acid $C_{16}H_{11}O_2N$ or $C_6H_5.C_9H_5N.COOH$.

Phenylcinchoninic Acid occurs in small, colourless needles or as a white or yellowish-white micro-crystalline powder; odourless or having a slight odour resembling benzoic acid, and a bitter taste. It is permanent in the air.

Insoluble in cold water; slightly soluble in cold alcohol, hot water or ether; readily soluble in hot alcohol.

It melts at about 210° with partial decomposition.

A saturated solution of Phenylcinchoninic Acid in hot diluted hydrochloric acid yields a precipitate of reddish-brown crystals with platinic chloride T. S. (U. S. P.).

Dissolve about 1 grm. of Phenylcinchononic acid in an excess of ammonia water, evaporate the solution to dryness on a water bath, or until free from the odour of ammonia, then dilute to 20 c.c. with distilled water, and filter. Separate portions of this filtrate yield a white flocculent precipitate with silver nitrate T. S.; a yellowish, flocculent precipitate with lead acetate T. S.; and a green flocculent precipitate with copper sulphate T. S.

No weighable ash remains on incinerating about 0.5 grm. of Phenylcinchoninic Acid.

Neocinchophen.—Ethyl-6-methyl-2-phenyl-quinolin-4-carboxylate, the ethyl ester of methyl-phenyl-quinolin-carboxylic acid.—$CH_3.C_9H_4.N.C_6H_5COOC_2H_5.6:2:4$. Neocinchophen was first introduced as novatophan.

Neocinchophen is a pale yellow, crystalline powder; odorless and tasteless; permanent in the air.

Neocinchophen is nearly insoluble in water and in dilute alkalies; soluble in hot alcohol and in strong acids; very soluble in ether and chloroform.

The m. p. of neocinchophen is not below 75°.

Boil 0.1 grm. of neocinchophen with 0.5 c.c. of sodium hydroxide solution and add 5 c.c. of iodine test solution. The odour of iodoform is apparent.

Dissolve 0.1 grm. of neocinchophen in 1 c.c. of sulphuric acid and add bromine water test solution drop by drop until an excess has been added. A yellow precipitate results.

Boil 0.1 grm. of neocinchophen with 1 c.c. of hydrochloric acid, filter while hot and add a few drops of platinic chloride test solution. A yellow, crystalline precipitate results.

Add a few drops of ferric chloride test solution to an alcoholic solution of neocinchophen (1–100). A yellow colour is produced (distinction from cinchophen, which produces a reddish brown colour).

Warm about 0.5 grm. of neocinchophen in 10 c.c. of ammonia water. The substance does not dissolve (distinction from cinchophen).

Incinerate about 0.5 grm. of neocinchophen, accurately weighed. Not more than 0.1% of residue remains.

Action and Uses.—Cinchophen and cinchophen derivatives stimulate the kidneys so as to have a selective action on the excretion of uric acid, which is increased considerably. Under a purin-free diet the amount of uric acid in the blood is reduced one-half; when exogenous purins are given, the total amount is rapidly excreted, so that the content of uric acid in the blood remains at normal or below. Its influence on uric acid excretion is stronger and is exerted more promptly than that of sodium salicylate.

Quinazolines

The quinazolines may be regarded as derivatives of quinoline formed by the replacement of the 3CH group by N.

A substituted dihydroquinazoline, namely, 3-phenyl-3:4-dihydroquinazoline,

$$C_6H_4\left\langle\begin{array}{l} N = CH \\ \qquad\quad | \\ CH_2.NPh, \end{array}\right.$$

has acquired some practical interest as the base of "*orexin*," a preparation said to have valuable tonic, stomachic and appetising properties, on which, however, some doubt has been thrown (*Pharm. J.*, 1890

kiil, **20**, 709, 825, 977; **21**, 43). The usual dose of orexin is from 2 to 10 gr.

Orexin, which occurs as a hydrochloride having the composition $C_{14}H_{12}N_2,HCl,2H_2O$, is prepared by acting on the sodium derivative of formanilide with *o*-nitrobenzyl chloride, and reducing the *o*-nitro-benzylformanilide thus obtained with tin and hydrochloric acid.

Orexin (hydrochloride) crystallises with $2H_2O$ in white needles, m. p. 80°. When kept in a desiccator for some time they become anhydrous, and then melt at 221°. Orexin has a bitter taste, and somewhat intense, burning after-taste. The powder induces violent sneezing. Orexin dissolves readily in water (13 pts.) and alcohol, but not in ether. On adding an alkali to the aqueous solution the free base is separated as an oil which becomes crystalline when kept, or as a white flocculent precipitate readily soluble in ether and chloroform. A solution of orexin yields with mercuric chloride a white precipitate soluble in hot water, and redeposited in white needles on cooling. Potassium dichromate gives a yellow precipitate soluble on heating, and redeposited, on cooling, in golden-yellow needles. Bromine-water is decolourised with formation of a yellowish amorphous precipitate. Orexin reduces potassium permanganate in the cold.

On heating orexin in a test-tube with about twice its measure of zinc-dust, the strong characteristic odour of phenyl-isonitrile is produced. On treating the residue with hydrochloric acid, and adding bleaching-powder solution to the filtered liquid, a blue coloration is obtained, owing to the previous formation of aniline.

Orexin tannate has been introduced for administering to children. It is a tasteless powder which becomes brown and acquires an unpleasant taste at 100° and decomposes completely at a higher temperature. It is almost insoluble in water, and only slightly soluble in alcohol and ether, but readily soluble in very dilute hydrochloric acid (0.3%) from which solution it is precipitated unchanged by strong acid and dilute aqueous alkali.

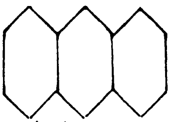
Orexin tannate gives a bluish-black coloration with iron salts and when treated with aqueous ammonia becomes clotted while the liquid assumes a wine-red coloration. A further test for orexin tannate is as follows: 0.5 grm. is dissolved in 3 c.c. of 30% acetic acid, rendered alkaline with sodium hydroxide and shaken with ether. The residue obtained by evaporating the ethereal extract is dissolved in concen-

trated sulphuric acid, addition of nitric acid to which should then produce a green coloration which frequently appears to be red on the edges. If the solution be diluted and treated with sodium hydroxide it should become yellow and yield a yellow precipitate.

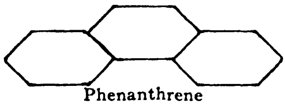
Orexin tannate when heated with zinc dust yields phenyl *iso*-cyanide, benzonitrile and aniline.

ACRIDINE AND ITS ALLIES

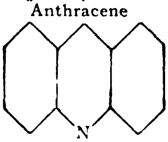
Acridine and its isomeride phenanthridine bear the same relation to anthracene and phenanthrene, respectively, that quinoline bears to naphthalene, and pyridine to benzene (compare). The following formulæ show their constitution and relationship to anthracene and phenanthrene:



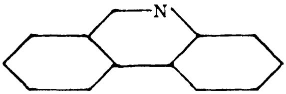
Anthracene



Phenanthrene



Acridine



Phenanthridine

Acridine. $C_{13}H_9N$

Acridine has been prepared synthetically by heating concentrated formic acid or chloroform with diphenylamine and zinc chloride, and also by various other reactions. Acridine is contained in coal tar, and may be extracted from the fraction boiling between 300° and 360°, or from crude commercial anthracene, by agitating it with dilute sulphuric acid, precipitating the acid liquid with potassium chromate, purifying the acridine chromate by recrystallisation, precipitating the base by ammonia, and recrystallising it from hot water. The hydrochloride may also be employed for the purification of acridine.

Acridine crystallises in colourless or brownish-yellow leaflets, broad needles or rhombic prisms, m. p. 110°; it sublimes in needles, distils without decomposition above 360°, and is volatile in steam.

Acridine is very slightly soluble in cold, but more readily in boiling, water, crystallising, on cooling, in long needles. It is readily soluble in alcohol, ether, benzene, carbon disulphide, etc.

Dilute solutions of acridine (and its salts) exhibit a strong blue fluorescence, which is green in more concentrated solutions, and disappears if they are very strong.

The most characteristic property of acridine is its intensely irritating effect on the skin and mucous membrane. Violent sneezing and coughing are produced by inhaling the smallest particle of the dust or vapour. The base and its salts attack the tongue even in minute quantities, and even very dilute solutions cause acute stinging when applied to the tongue or skin.

Acridine has been employed as an insecticide, and compositions containing it have been patented for coating the bottoms of vessels. It is highly probable that the preservative properties of coal-tar creosote oil are partially due to the presence of acridine.

Acridine is a very stable substance. Sulphuric acid has no action upon it, except at a very high temperature, and potassium hydroxide does not react below 280°. Concentrated nitric acid converts acridine into nitro-derivatives. Most other oxidising agents act with difficulty or not at all on acridine, but by the action of potassium permanganate it is converted into *quinoline-2:3-dicarboxylic* or *acridinic acid.* This substance crystallises with $2H_2O$ in slender needles, decomposes at 120–130°, and is sparingly soluble in water.

The addition of a 10% solution of cobalt nitrate to a boiling solution of 1 grm. of acridine in 500 c.c. of bleaching powder solution (1:5) and subsequently boiling for one hour leads to the formation of 9-*acridone,* stout, yellow needles, m. p. 354° (decomp.).

Salts of Acridine

Acridine is a feeble base. It forms no carbonate, and its salts are more or less decomposed by boiling with a large quantity of water.

Acridine hydrochloride, $C_{13}H_9N,HCl,H_2O$, forms yellow plates. The solution in water exhibits a bluish-green fluorescence and gives a yellow crystalline precipitate, m. p. 235°, of the *mercurichloride,* $(C_{13}H_9N,HCl)_2,HgCl_2$, on the addition of mercuric chloride. With platinic chloride it yields the *platinichloride,* $(C_{13}H_9N)_2,H_2PtCl_6$, in minute, sparingly soluble, yellow needles.

Acridine nitrite, $(C_{13}H_9N)_2, HNO_2,3H_2O$, obtained by the interaction of acridine hydrochloride and sodium nitrite, forms long, yellow, silky needles, m. p. 151°. It loses $2H_2O$ at 70–80°.

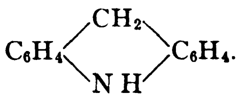
Acridine sulphite, $(C_{13}H_9N)_2,H_2SO_3$, is precipitated in yellowish-red or brownish needles, very slightly soluble in water, on mixing

solutions of sodium sulphite and acridine hydrochloride, and adding hydrochloric acid.

Acridine Picrate, $C_{13}H_9N,C_6H_3O_7N_3$, is obtained as a canary-yellow precipitate, consisting of microscopic, yellow, prismatic needles with a faint green reflex; it commences to decompose at 208°. The picrate is almost wholly insoluble in cold water; 10 c.c. of the saturated solution in alcohol and benzene at 17.5° contain 0.004 grm. and 0.001 grm. of the salt, respectively. Acridine has been suggested by Anschütz (*Ber.*, 1884, **17**, 438) as a suitable reagent for the estimation of picric acid, the hydrochloride being used as a precipitant for metallic picrates, and a solution of the free base in benzene for the picric acid compounds of hydrocarbons.

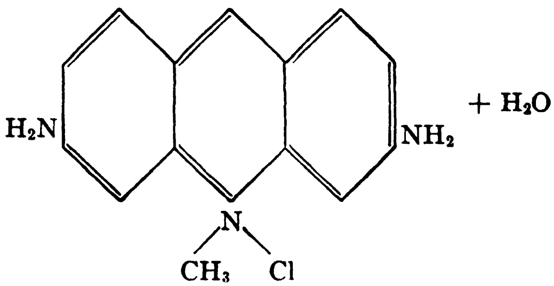
Conversely, in the absence of substances forming picrates soluble with difficulty in benzene or water, acridine may be estimated by precipitating and weighing as the picrate.

Hydroacridine. Dihydroacridine



This substance is formed (together with a white substance insoluble in alcohol) by the reduction of acridine in alcoholic solution by sodium amalgam. It forms prisms, m. p. 169°, insoluble in water, slightly soluble in cold alcohol, very soluble in hot alcohol or ether. It dissolves in concentrated sulphuric acid, and is precipitated unchanged on dilution with water. Argentic and cupric oxides reconvert it into acridine. Hydroacridine is the analogue of piperidine (page 642) and tetrahydroquinoline (page 656).

Acriflavine.—Acriflavina.—3:6 diamino-10 methyl-acridinium chloride = $C_{14}H_{14}N_3Cl.H_2O$.



Acriflavine is a brownish-red, odourless, crystalline powder. It is soluble in less than 2 parts of water and in alcohol, forming dark red solutions which fluoresce on dilution and which have a bitter taste. Nearly insoluble in ether, chloroform, liquid petrolatum, fixed oils and volatile oils.

An aqueous solution of acriflavine (1:250) is neutral to litmus paper (it dyes the litmust paper yellow).

Add a few drops of hydrochloric acid to an aqueous solution of acriflavine, which is sufficiently dilute to be fluorescent. The fluorescence disappears, but partially reappears on further dilution with water.

Add 2 drops of sulphuric acid to about 1 c.c. of an aqueous solution of acriflavine (1:250) and agitate the mixture. An orange-red, crystalline precipitate is produced. Under the microscope the crystals are seen to be mostly long needles or prisms arranged in sheaf-like or brush-like forms.

An aqueous solution of acriflavine (1:250) gives a precipitate with silver nitrate solution (distinction from proflavine).

An aqueous solution of acriflavine (1:250) does not give a precipitate with barium chloride solution (distinction from proflavine).

An aqueous solution of acriflavine (1:250) does not give a precipitate with formaldehyde solution (distinction from proflavine, which gives a brown precipitate).

Add 2 drops of diluted hydrochloric acid to 5 c.c. of an aqueous solution of acriflavine (1:250) and immediately add 2 drops of sodium nitrite solution (1:10). A violet colour is produced. By the further addition of an excess of sodium nitrite solution, a violet precipitate is formed and, after a few minutes, the colour of the solution becomes cherry-red. This may be best observed after filtration (distinction from proflavine, the filtrate from which is colourless).

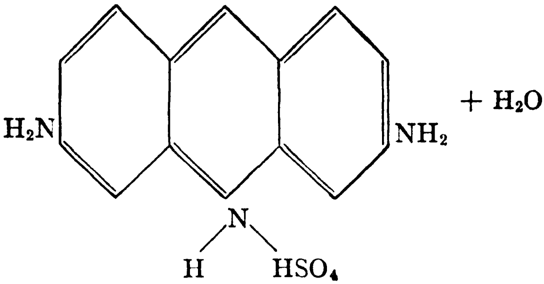
An aqueous solution of acriflavine (1:250) gives an orange precipitate with sodium hydroxide test solution (distinction from proflavine, which gives a yellow precipitate).

Incinerate about 1 grm. of acriflavine, accurately weighed. The ash amounts to not more than 1 per cent.

Dissolve about 1 grm. of acriflavine, accurately weighed, in 250 c.c. of warm water, collect the insoluble matter, if any, in a weighed Gooch crucible, wash the insoluble matter with hot water, dry and weigh the residue. The insoluble matter amounts to not more than 1%.

Dry about 1 grm. of acriflavine, accurately weighed, to constant weight at 100°. The substance loses not more than 10% of its weight.

Proflavine.—Proflavina.—3:6 diamino acridine sulphate = $C_{13}H_{11}N_3H_2SO_4.H_2O$.



Proflavine is a reddish-brown, odourless, crystalline powder.

Proflavine is soluble in water and in alcohol, forming brownish solutions which fluoresce on dilution. It is nearly insoluble in ether, chloroform, liquid petrolatum, fixed oils and volatile oils.

An aqueous solution of proflavine is neutral to litmus paper (the solution dyes the litmus paper yellow).

Add a few drops of hydrochloric acid to an aqueous solution of proflavine, which is sufficiently dilute to be fluorescent. The fluorescence disappears, but partially reappears on dilution with water.

Add 2 drops of sulphuric acid to about 1 c.c. of an aqueous solution of proflavine (1:250), and agitate the mixture. A brown, crystalline precipitate is produced. Under the microscope the crystals are seen to be mostly prismatic needles.

An aqueous solution of proflavine (1:250) gives a precipitate with barium chloride solution (distinction from acriflavine).

An aqueous solution of proflavine (1:250) gives no precipitate with silver nitrate solution (distinction from acriflavine).

Add a few drops of formaldehyde solution to 5 c.c. of an aqueous solution of proflavine (1:250). A brown precipitate is given (distinction from acriflavine, which remains clear).

Add 2 drops of diluted hydrochloric acid to 5 c.c. of aqueous solution of proflavine (1:250), and immediately add 2 drops of sodium nitrite solution (1:10). A violet colour is produced. On the further

addition of sodium nitrite solution a brownish violet precipitate is formed, and, after a few minutes, the solution becomes colourless. This may be best observed after filtration (distinction from acriflavine, the filtrate from which becomes cherry-red).

An aqueous solution of proflavine (1:250) gives a lemon yellow precipitate with sodium hydroxide solution (distinction from acriflavine, which gives an orange precipitate).

Incinerate about 1 grm. of proflavine, accurately weighed. The ash amounts to not more than 1%.

Dissolve about 1 grm. of proflavine, accurately weighed, in 250 c.c. of warm water, collect the insoluble matter, if any, in a weighed Gooch crucible, wash the insoluble matter with hot water, dry and weigh the residue. The insoluble matter amounts to not more than 1 per cent.

Dry about 1 grm. of proflavine, accurately weighed, to constant weight at 100°. The substance loses not more than 10% of its weight.

Actions and Uses.—Acriflavine and proflavine have been found to be strongly antiseptic; this action does not appear to be weakened in the presence of serum. In the treatment of wounds, it is claimed that acriflavine and proflavine are comparatively free from toxic or irritant action on living tissues, and that they do not inhibit appreciably the phagocytic action of the leucocytes on the healing process. Acriflavine is claimed to exert a specific bactericidal action on the gonococcus. The evidence indicates that acriflavine has a greater antiseptic action than proflavine, though the action of acriflavine is slower.

Phenanthridine

Phenanthridine crystallises in slender needles, m. p. 104°, and distils above 360°. The vapour when inhaled induces violent sneezing. Aqueous solutions exhibit a blue fluorescence. It is thus seen that phenanthridine presents the closest resemblance to acridine; it may be distinguished from the latter substance, however, by adding sodium sulphite to a solution of the hydrochloride, containing excess of hydrochloric acid; phenanthridine does not yield a precipitate whilst acridine gives a precipitate of reddish-brown needles. The *mercurichloride*, $C_{13}H_9N,HCl,HgCl_2$, crystallises in small prisms, m. p. 197°.

A boiling solution of phenanthridine in bleaching powder solutions, when treated with cobalt nitrate, yields *phenanthridone* which crystallises in long, silky needles, m. p. 293° (corr.).

The analyst is referred to Thorpe's Dictionary of Applied Chemistry, which includes the best articles in English on all of these subjects, and to Teerfarbenfabrikation and verwandter Industriezweige by Friedlaender, if he wishes to go to the patents.

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